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Report of program activities.

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ANNUAL REPORT

NATIONAL CANCER INSTITUTE

Office of the Director

1960

This year, the twenty-third year of the National Cancer Institute, stands out as a year of change--almost as the passing of an era. The Institute underwent its second major reorganization. There was an almost complete change of top management. A number of key individuals retired or moved to new posts.

The budget passed the $100 million level and more than a dozen new major programs were launched. As might be expected, the year-end found much unfinished business and many unsolved problems. Recruitment of program leaders and provision of working space still loomed as tough challenges.

But despite these many changes and problems, solid scientific progress was made and plans were laid for a broad, intensive research attack on cancer problems at the basic, applied and developmental levels.

Perhaps of greatest long-range significance was the creation of a program of broad support to clinical cancer research centers. NCI took the first steps to provide $5.5 million for the creation of a few such centers along with $5 million for construction of cancer research facilities.

The Institute's field studies were reorganized along two principal lines: 1) elucidation of specific factors in the occurrence of cancer, either by studying groups exposed to heavy concentrations of suspected carcinogens, or by retrospective case-control analyses of specific types of cancer; and 2) study of large population groups and environments as "human population laboratories." Plans were also laid for studies of cancer in animals in their natural environment, particularly from the standpoint of reaction to environmental or infectious agents.

Planning for the basic requirements of a long-range and broadly supported program of virus-cancer research occupied a large share of attention during the year. Activity in this field, which reached a level of more than $5,000,000 in grant support in 1960, produced heavy demands for vital research materials. Steps to satisfy these needs were taken with the establishment of a committee to develop criteria for certifying and preserving strains of mammalian cells of interest in cancer research. More than a dozen laboratories throughout the country became interested in assisting with this effort, and the American Type Culture Collection in Washington, D. C. agreed to serve as a repository and distributing center for standard cell strains. The Institute awarded a contract to

the University of Kansas Medical Center to develop diagnostic reagents which will identify known viruses of the human digestive tract, taking advantage of laboratory resources previously developed by the National Foundation for research on a polio vaccine.

In the chemotherapy program, support of pre-clinical work-up for active drugs was increased, and that of screening and related preliminary-type activities was reduced. This was done to compensate for increased costs of research and testing in the current market, and to support pre-clinical pharmacology and related work in order to make a greater volume of compounds ready for trial in man.

The Institute also geared its activities to participate in several integral programs which also involve other Institutes and Divisions of NIH. One of these is of an international nature and is presently administered through the National Institute of Allergy and Infectious Diseases. It contemplates fuller use of resources, both in scientific manpower and fields of study, available in other countries. Another is the newly authorized career research professorships which are expected to provide career stability for many of the scientists participating in the multi-discipline approach to cancer research.

The full impact of implementing new programs and expanding existing operations, such as cancer centers, environmental cancer studies, virus and diagnostic research, was just beginning to be felt in 1960. A partial assessment of this was made late in the year when the Director, the Executive Officer, and the Associate Director for Grants and Training visited several medical schools and other institutions to discuss the non-matching grant construction and center programs, and policies and practices which would best meet the needs of recipient institutions and program objectives.

Reorganization of the Institute to redirect operations along the new approaches accomplished a re-grouping of many activities under three newly designated Associate Directors.

Grants and training activities were consolidated in several branches under Dr. Ralph G. Meader as Associate Director. This includes the cancer centers program and, for the time being, the program on viruses and cancer. Field investigation grants, the modified traineeship program and the teaching grant program are also being transferred to this organizational area. Dr. Meader had been Chief of the Research Grants Branch since 1948.

Studies in biometry, epidemiology, environmental cancer and diagnostic research, including field investigations, were grouped under Dr. Michael B. Shimkin as Associate Director. These activities will be administered through four branches. The Field Investigations and Demonstrations Branch, which formerly handled some of these operations, was abolished. Its Nursing Section was transferred to the Bureau of

State Services. Plans have been made to provide laboratory back-up for field studies and to foster closer working relations between field work and intramural research activities. Dr. Shimkin had been Chief of the Biometry Branch since 1954.

Dr. Stuart M. Sessoms, Chief of the Cancer Chemotherapy National Service Center since 1958, was designated Associate Director in Charge of these activities.

Full responsibility for administration of clinical research at the National Cancer Institute was assigned to Dr. Charles G. Zubrod as Clinical Director. Dr. Carl G. Baker, Assistant Director, was given responsibility for nonclinical research at the Institute. Dr. Eli M. Nadel, formerly Assistant to the Associate Director for Intramural Research of the National Institutes of Health, was appointed an Assistant Director of the Institute.

As of July 1, 1960 Dr. Kenneth M. Endicott, formerly Associate Director, National Institutes of Health, was appointed Director of the National Cancer Institute. He succeeded Dr. John R. Heller, Director since 1948, who retired to become President of Memorial Sloan-Kettering Cancer Center in New York.

Other principal staff changes in 1960 included the transfer of Dr. G. Burroughs Mider, Associate Director in Charge of Research, to fill the new post of Director of Laboratories and Clinics, National Institutes of Health. Three key scientists retired: Dr. Alexander H. Gilliam, Assistant Chief of the Field Investigations and Demonstrations Branch, who joined the Faculty of Johns Hopkins University Medical School; Dr. John E. Dunn, who remained with the California State Health Department where he had been on detail for several years; and Dr. Herbert Kahler, Head of the Physical Biology Section, Laboratory of Physiology. Dr. Howard B. Andervont, a member of the original research staff of the National Cancer Institute, relinquished his post as Chief of the Laboratory of Biology, and although eligible for retirement, arranged to continue his research in the Laboratory and accepted appointment as Scientific Editor of the Journal of the National Cancer Institute to succeed Dr. Shimkin.

Provision was made for a Special Assistant to work with the Director in the development of the various new programs facing the Institute. The appointee, Dr. Margaret H. Sloan, who was scheduled to join the Institute's staff around February 1, 1961, will be concerned initially with programs in the international field.

A new function was established in the Office of the Director with the appointment of a Financial Projects Officer. In this position Thomas J. Cassidy will be responsible for evolving financial mechanisms and making analyses of program controls for the new activities being undertaken by the Institute, in addition to normal budget formulation processes.

Technical guidance of related activities administered by the Cancer Control Program of the Bureau of State Services continued to be a function of the Director, National Cancer Institute. The Cancer Control Program gave special priority to early case-finding of uterine cancer, stressing the importance of exfoliative cytology as a detection technique and an aid to diagnosis. The community demonstration project grant program was emphasized, and expansion of State cancer control programs was encouraged through the formula grants which were adjusted to allow a minimum of $25,000 to each State.

A reorganization directive by the Surgeon General providing for close coordination between the National Cancer Institute and the Cancer Control Program for the preparation and dissemination of materials for public information and education purposes was implemented with the establishment of a Joint Committee on Cancer Information. This group, co-chaired by James F. Kieley, Information Officer of the National Cancer Institute, and Dr. Lewis C. Robbins, Chief of the Cancer Control Program, will study research progress and cancer control developments in the light of planning for public information and education activities, and will facilitate a continuous exchange of information between the Institute and the Cancer Control Program.

Steadily increasing activities over the past several years, and preparations for even larger programs have made additional laboratory and office space an urgent requirement. While personnel have been moved about between the NIH campus and rented quarters in Silver Spring and Bethesda, plans have been developed for additional space in the new NIH General Office Building now under construction, and in a new National Cancer Institute Building which is being planned with funds from a $700,000 item for this purpose in the 1961 appropriation.

The Institute has been allocated one and a half floors (17,500 square feet) of space in the new General Office Building scheduled to be ready for occupancy in the fall of 1961. Request has been made for an additional half-floor.

The proposed National Cancer Institute Building, which will cost about $10,000,000, is estimated to provide 135,000 net square feet of floor space and will be ready in 1964 if the funds for construction are appropriated for fiscal 1962.

Reports on several activities administered as a part of the Office of the Director follow.

Office of Information and Publications

Office of the Director

National Cancer Institute

Program Activities Report, Calendar Year 1960

The organization and interpretation of data on the progress of research and related programs for public information and administrative purposes was continued as a major activity of the Office of the Director, and provision was made to expand these operations to meet the requirements of the Cancer Control Program of PHS.

As an implementation of the Public Health Service reorganization, the Surgeon General directed that close coordination be effected between the information activities of the Cancer Control Program and the National Cancer Institute. This was accomplished through the establishment of a Joint Committee on Cancer Information which will maintain a constant review of all public information activities in use or planned by either agency in terms of their impact and effect on the responsibilities and activities of the other. The Committee will also study research progress and program developments in both areas in the light of planning for public information activities, and will facilitate a continuous exchange of information between the Institute and the Cancer Control Program. The Cancer Control Program will rely heavily on the technical facilities of the NCI Office of Information and Publications for the production and distribution of public information and educational materials.

With the appointment of the NCI Information Officer as a member of the Public Information Committee of the American Cancer Society, an additional basis was provided for cooperation between the Society and the Public Health Service in the development of information and education programs in the public interest.

Encouragement of activities for the improvement of science teaching in secondary schools as a measure looking toward relief of the scientific manpower shortage was continued. The Information Officer participated in a series of conferences with the Training Committee, the National Advisory Cancer Council and officials of the National Science Teachers Association and others preliminary to approval of a grant of $239,700 to the NSTA for a research project on the preparation of educational films for the junior high school level. He also participated in an organization conference of the Advisory Board for this project with members of the faculty of the University of Oklahoma where the project will be carried out.

Arrangements were made for the National Science Teachers Association to make classroom evaluations over a school year of the illustrations and detailed lecture notes entitled "Let's Talk About Cancer" which were prepared by the Office of Information and Publications for use with lay audiences. If the material proves to be useful for teaching purposes, NSTA will arrange to have it adapted for film strips and other forms and made available to schools throughout the country.

The Information Officer participated in a conference of educators and representatives of voluntary and commercial organizations supporting the Future Scientists of America program of the NSTA, for which several publications will be produced out of a grant of $7,652 awarded by the National Cancer Institute.

The Information Officer participated in the judging of 400 entries in the STAR-60 project (Science Teacher Achievement Recognition project for 1960) conducted by the National Science Teachers Association under a grant of $35,650 from the National Cancer Institute.

The Office of Information and Publications also developed for the National Science Teachers Association a detailed proposal for a research project on the development of improved techniques for utilizing current scientific information in the preparation of laboratory demonstrations. The NSTA is seeking grant support elsewhere for a project along these lines to be conducted by a research team of teachers and scientists.

The Information Officer and staff participated in several conferences and discussions leading to approval of a grant of $65,000 to the University of Minnesota for the production of a series of television programs on cancer control. At the year's end preparations were being made for a conference early in January between the Joint Committee on Cancer Information and faculty members of the University of Minnesota for discussion of final details before work on the project is started.

The resources of the Office of Information and Publications were devoted largely to preparations for and assistance with the Fourth National Cancer Conference which was held at the University of Minnesota September 13-15. This included the installation of more than 30 scientific exhibits, arrangements for a film program, and cooperation with the American Cancer Society in operating press services both on campus and at the headquarters hotel in Minneapolis.

At the close of the year the Director had under advisement a recommendation from the Information Officer for a change of Institute policy in the handling of requests for the testing or recognition of unproved cancer treatments and purported cures. This will be considered in relation to the screening activities of the Cancer Chemotherapy National Service Center and other aspects of the Institute's program.

The Information Officer attended the first Public Relations Institute conducted by the National Publicity Council of Health and Welfare Agencies in New York in June.

Research and Program Reports Section -- Materials prepared in connection with the Congressional Hearings on the NCI appropriation for FY 1961 included: the summary of the Director's Opening Statement, drafted the previous year, a special report on the chemotherapy program, and the NCI portion of the House Committee Report. Materials prepared for Hearings on the FY 1962 Budget included a draft of the Director's Opening Statement and "Highlights of Progress in Research on Cancer, 1960."

In response to requests from Members of Congress, the following materials were prepared: statements on NCI grants for research in Rhode Island for Representative Fogarty, and on grants for research in Wisconsin for Representative Laird; a speech on cancer research for Senator Randolph of West Virginia; and speech materials for Representative Fogarty.

The weekly report to the Director, NIH, contained 77 items, including 25 program highlights and 52 research highlights.

For the Fourth National Cancer Conference in Minneapolis, September 12-15, which was sponsored jointly by the National Cancer Institute and the American Cancer Society, five press releases were written, and issued by the Conference. Texts of two exhibits and a 10-page booklet to accompany the exhibit, "Changing Concepts Concerning Cancer," were prepared.

During the year, detailed plans were drawn for the writing and production of a series of lay information pamphlets intended primarily for use in answering public inquiries. The first publication in this series, "Treating Cancer," was completed. Companion publications now in preparation are: "Cancer Research," "Cancer Cause and Prevention," "Diagnosing Cancer," and Hodgkin's Disease." Comprehensive revision of the National Cancer Institute Brochure was begun.

Nine speeches and articles were prepared for the Director, and summaries of three were provided for press purposes at meetings of the Texas State Medical Association, the Kentucky Medical Association, and the Southeastern States 1960 Cancer Seminar. One speech was prepared for the Deputy Surgeon General. The Section prepared press summaries of speeches delivered by the Associate Director for Cancer Chemotherapy to the American Chemical Society and the American Cancer Society Science Writers' Seminar.

Three general press releases, two professional releases, and an announcement were issued. Three summaries were prepared of scientific papers presented by staff members to the Federation of American Societies for Experimental Biology, the American Association for Cancer Research, and the Fifth International Congress on Nutrition.

Assistance in their assignments was given to 22 science writers. They received source material on cancer research, statements for attribution to the Director and other staff members, and appointments for interviews with NCI scientists and officials.

Miscellaneous materials prepared during the year included: the NCI portion of the 1960 Annual Report of the Secretary, DHEW; the President's Proclamation and the Secretary's statement announcing Cancer Control Month; three one-page reports to the President's Scientific Advisor; a report on NCI leukemia research for the Vice-President; background material on the worldwide cancer problem for a United States Information Service film; a four-page background statement on virus-cancer research for possible use by the Secretary at a press conference; and a background statement on theories of cancer research for the Director's use on a television broadcast.

In addition, a statement summarizing conferences and correspondence concerning Krebiozen was prepared. Source materials for the NCI portion of the Clinical Center exhibit at the American Medical Association meeting were drawn up. A description of NCI purpose, function, and areas of research was prepared for inclusion with a listing of scientific publications in a National Science Foundation report. Background information was provided for the revision of the National Health Education Committee publication, "What are the Facts on the Major Killing and Crippling Diseases in the United States." Several stories were contributed to the NIH Record.

Section members organized and staffed the press room at the American Association for Cancer Research meeting in Chicago and the Fourth National Cancer Conference. Section members also attended the American Medical Association meeting in Washington, D. C.; and the annual meeting of the American Association for the Advancement of Science and a Symposium on the Phenomena of Tumor Viruses, in New York.

Recruitment for new staff members was successfully completed by the appointment of three writers. They replaced two writers who had resigned and filled one vacancy that had existed for more than a year.

Information and Education Section - Materials prepared included releases on new appointees to the National Advisory Cancer Council and on one new lay education pamphlet and documents requesting Bureau of the Budget renewal of authorization for continued publication of the Journal of the National Cancer Institute.

Arrangements were made for the production of several sets of 38 slides for use with a speech outline, "Let's Talk About Cancer." Information on the availability of this material for presentation to lay audiences was circulated among the Institute staff.

The Section Head received and made appropriate appointments with staff members for lay and professional visitors to the Institute from this country and abroad. Arrangements were made for 10 professional visitors, one lay and one professional group. In the case of one professional visitor from Geneva -

Chief, Cancer Control Program, World Health Organization - appointments were made with 23 scientists at 16 institutions in various parts of the United States.

Text was prepared or revised and preliminary layouts arranged for four new exhibits for initial showing at the Fourth National Cancer Conference in Minneapolis in September. Liaison was maintained by the visual information specialist with the Medical Arts Section, NIH, and Design and Production Incorporated, Alexandria, during design and construction. One exhibit, on the theme of the conference, "Changing Concepts Concerning Cancer," was sponsored jointly by NCI and the American Cancer Society, co-sponsors of the conference. Two exhibits were on the viral etiology of cancer, and the worldwide geographical distribution of cancer, respectively. The fourth exhibited leading scientific journals on cancer and both lay and professional publications. Following the Minneapolis showing, the exhibits were shown at the American Medical Association clinical meeting in Washington, and in the lobby of the DHEW building.

In connection with the Minneapolis conference, the visual information specialist also handled all arrangements for the showing of 30 medical and scientific exhibits. This entailed invitations and correspondence with medical schools and other organizations selected to contribute exhibits and films, close coordination with the American Cancer Society and the University of Minnesota, and arranging suitable booths and details of installation. Medical and health education films were also selected and arrangements made for continuous showing during the Conference. A book exhibit was arranged in cooperation with leading medical book publishers and the University of Minnesota bookstore.

Text and suggested illustrations were submitted to PHS for use as part of an exhibit in Rome during November and December. Visuals and other needs such as microscopes and slides were also supplied.

Other materials produced included charts for staff use during the Congressional hearings, photographs of research at NCI, and lantern slides for use of the professional staff. Appropriate photographs and captions were furnished at requests of reporters, to accompany story material, or to illustrate articles prepared in OIP.

Inquiries from the public and Congressional sources dropped slightly from 1959, although the volume was still high enough to require occasional overtime in the two-person public inquiries unit. Replies to public inquiries totaled 1,225, and those to Congressional inquiries, 101. Of the latter, 43 were on the subject of Krebiozen, representing a 57 per cent decrease from 1959.

Requests to test proposed cancer cures continue to come in, with an increasing number from foreign countries.

Informational literature distributed with letter replies totaled over 3,800 pieces. Telephone inquiries to the unit totaled 700, or an average of 13.4 a week.

A total of 60 items were processed through clearance, including 46 speeches, articles, press releases, special statements, and other materials produced by the information staff. The remainder were papers submitted for clearance by other Institute staff members.

Reference and Distribution Section -- The screening of selected journals for papers published by NCI staff personnel or grantees, for use by OIP writers, continued. During the year a total of 1,284 issues of journals, or an average of 107 per month, were screened from which a total of 1,465 reference cards were made. From these cards was compiled a list of 1,300 titles of papers published during Fiscal Year 1960 by Institute staff members, grantees and contractors. This list is being prepared for printing as a PHS publication.

A bibliography on arsenical cancer was prepared for a PHS Committee on Food Additives, and integrated with a large reference list furnished by Dr. W. C. Hueper. The polyoma virus bibliography, prepared last year, was updated, and a list of NCI scientific publications was prepared for inclusion in a National Science Foundation report.

Bulk distribution of lay and professional publications and reprints on various aspects of cancer research and related activities totaled 52,371. One new lay education pamphlet, "Treating Cancer," was released, with wide distribution.

Individual requests for educational material which come from students, teachers, professional and service organizations and the general public, and which do not generally require letter replies, totaled 2,113. In response, 10,172 pieces of printed material were sent.

Distribution of press releases and announcements to science writers and professional journals totaled 3,250, and monthly summaries of papers appearing in the Journal of the National Cancer Institute, which are mailed to science writers and professional journals, 8,500. Film bookings for diagnostic and lay films numbered 132, with 78 referrals to the regional offices for "Challenge: Science Against Cancer." Nine exhibits were booked for 17 showings.

Eighteen-hundred and twenty-three issues of seven newspapers were screened in this Section, and in the Information and Education Section. Cancer-related articles were clipped, circulated and filed for further reference.

Program Activities Report

Calendar Year 1960
Journal of the National Cancer Institute

The Board of Editors reviewed 636 items for publication this year. Of this number, 235 were manuscripts by NCI staff for publication in other scientific or medical journals--232 were approved, 1 was withdrawn, 2 are pending; 228 (49 by NCI staff and 179 by authors outside) were manuscripts submitted for publication in JNCI--140 were approved, 30 were rejected, 1 was withdrawn, and 57 are pending. There were 173 abstracts and book reviews. All were approved.

Volumes 24 and 25 contained 2,940 printed pages. Papers awaiting publication comprise issues scheduled through April 1961, each of 250 pages.

Three NCI Monographs were published:

No.2, On Normal and Abnormal Differentiation and Development, 188 pp.;
No.3, Conference on Experimental Clinical Cancer Chemotherapy, 306 pp.;
No.4, Symposia Tumor Viruses, 376 pp.

Two additional Monographs have been received for publication in 1961.

The Board of Editors was responsible for review of papers given at the Fourth National Cancer Conference, September 13-15, Minneapolis, Minnesota, and for arrangements regarding publications of these papers as a book with J. B. Lippincott Company, Philadelphia.

Dr. Michael B. Shimkin, Scientific Editor, was succeeded by Dr. Howard B. Andervont, January 1, 1961.

NATIONAL CANCER INSTITUTE

CANCER CHEMOTHERAPY NATIONAL SERVICE CENTER

Cancer Chemotherapy Program

The Cancer Chemotherapy National Service Center was established in 1955 to accelerate research and speed the introduction of promising agents into clinical trial. It has accomplished both goals. Cancer chemotherapy research now constitutes the largest and most comprehensive single approach to solution of the cancer problem; more drugs are being evaluated in the clinic today than ever before.

In achieving these goals, the Center has established effectual mechanisms for obtaining large amounts of new materials -- chemical synthetics, endocrine substances, antibiotics and other natural products -- to test for antitumor activity. Complex screening systems, provided by contracts, are in operation selecting potential materials for cancer therapy. Basic and applied studies are supported, investigating other major approaches toward the discovery of chemotherapeutic agents. "Pipe lines" have been established to carry interesting materials through toxicology and pharmacology to preliminary and definitive clinical trial in a large coordinated clinical evaluation program.

The clinical phase of the Service Center's program has expanded at an unprecedented rate. A total of 50 new compounds was added to the 109 being evaluated a year ago for anticancer activity. Of the 159 drugs now in clinical trial, only 31 were in use prior to the establishment of the chemotherapy program almost six years ago. Scientists in more than 150 hospitals throughout the United States and Western Europe are conducting some 200 clinical studies, which to date have involved more than 16,500 patients. (See Appendix I for listing of Cooperative Clinical Studies)

While sound scientific patterns have been achieved for developing agents for cancer chemotherapy, many complex problems remain unsolved that are fundamental to the effectual operation of the program.

One of the major problems is the selection of a laboratory screening system with specificity for a large spectrum of human malignancies, while still remaining a manageable operation. Since cancer now is generally conceded to be a generic class of diseases -- possibly numbering into the hundreds -- it is important that as many types as possible be represented in a screen for therapeutic agents.

Another important problem of the preclinical activities is validation of the laboratory testing systems. Until laboratory results are correlated with results in the clinic, the effectiveness of systems used in the laboratory to establish the relative therapeutic value of groups

of related active materials will not be known nor will it be known for which of the many human malignancies a given test system is predicting beneficial activity.

The answer to these and other perplexing questions will only be secured by augmented, properly coordinated studies in the cooperative clinical program. Many preliminary studies need to be undertaken in as broad a spectrum of human cancers as possible to secure adequate trial: of new types of chemical structures in cancer chemotherapy, of analogs of existing agents evincing greater activity in the laboratory, and of those agents demonstrating different mechanisms of action. Other quantitative studies are required to select the agents of choice in specific human malignancies and to develop better methods for determining meaningful criterial of response to therapy.

The major part of the Chemotherapy Service Center's preclinical program is carried out under contracts with pharmaceutical firms, research institutions, colleges, and universities. (See Appendix II for complete listing of active contracts) Contracts are established for drug procurement and synthesis, the primary and secondary evaluation of drugs, studies to refine chemotherapy methodology, pharmacological and toxicological evaluations, and mechanical data processing activities. (For allocations of funds to the various areas of research see Appendix III) Contracts are also utilized to collect statistical data from more than 200 hospitals in various parts of the United States. A variety of medical institutions are represented including community hospitals, state and federal hospitals, university hospitals, and a cancer research center. The program is designed to provide information on the efficacy of various methods of treatment, primarily in terms of survival. Collaborative studies with tumor registries in Denmark, England, Finland, France, and Norway are in progress.

The cooperative clinical program is financed largely by Research Grants (Veterans Administration clinical trials are funded by direct transfer). Transfer of fund agreements have been effected with the Veterans Administration, Department of the Army, Department of Agriculture, and the Food and Drug Administration for performance of various Center activities. (See Appendix IV)

Current obligations for support of active contracts, plus funds needed to activate newly approved contracts, amount to $21,380,000. This is about $160,000 less than the total amount allocated for contracts in the Center's 1961 budget. However, normally rising expenses tend to raise the cost of contract work about five percent a year. Therefore, it has been necessary to cut back the contract activity in order to effect a $1,000,000 reduction. Economies have been chiefly in the area of primary screening; and where possible, funds have been shifted to secondary evaluation, pharmacology, and other preclinical activities, which have become more important in the past several years as a result of the expansion in clinical research.

The total appropriations for fiscal year 1961 for the programmed activities of the Center appears in Table 1. A proposed transfer of $1,300,000 of Grant's funds to Contracts currently underway is, in the main, to finance projects to prepare the increasing number of confirmed active compounds for clinical evaluation and to expand efforts in the secondary evaluation of compounds which have passed the criteria for active agents.

TABLE 1

CANCER CHEMOTHERAPY NATIONAL SERVICE CENTER

RECAPITULATION OF FY 1961 FUNDS

	Appropriation
Chemotherapy Contracts	$21,538,000
Clinical Grants	5,300,000 1/
Center Operations	1,444,000
TOTAL	$28,282,000

1/ Arrangements are underway to transfer $1,300,000 to chemotherapy contracts in order to activate new contracts that have been held pending availability of funds.

Program Developments

During 1960 a comprehensive program review was completed; priorities were established and plans were laid for furthering program objectives. Implementation of these plans is discussed in the following description of major program developments.

Drug Procurement and Development. Materials to be screened for antitumor activity are obtained by off-the-shelf purchase or gift from educational institutions, research organizations and manufacturing concerns; or from contracts established for the development of synthetics related to active leads found in the primary screen and the production of antibiotic materials. Such materials are flowing to the Center at the rate of about 50,000 annually and include synthetic chemicals, hormonal substances, plant and animal extracts, and antibiotic culture filtrates.

Pilot plant and preparative laboratories have been established to supply the large quantities of active compounds required for preclinical and clinical evaluation studies. Activities have been expanded for the preparation of drugs into dosage forms for clinical trial, since the physical and chemical properties of the new antitumor drugs frequently require special formulation, and facilities have been established for control of the quality of these materials furnished for trial.

Primary Screening. The three tumor primary screen of the CCNSC has now been operating at full strength for approximately three years and during this period about 40,000 synthetics and 60,000 antibiotic substances have been screened. Many substances demonstrating antitumor activity have been encountered. Of major importance, however, is that among these active materials are about twenty-two unique substances, of comparatively new chemical structure, not previously known to be active in tumor systems. Eleven of these are already in preliminary clinical trial and the remainder are undergoing various stages of development preparatory to trial.

Careful consideration of the screening program has indicated that important opportunities are being overlooked in limiting the primary screen to three mouse tumors; that, quite possibly, the addition of new types of animal tumors now becoming available would uncover materials with different spheres of action in human malignancies which would be of value in the treatment of tumors including those heretofore unresponsive to chemotherapeutic agents.

It has consequently been decided to broaden the primary screen by including selected new mouse, rat, and hamster tumors. It is planned that this change will be accomplished without substantial increase in the over-all size of the primary screening operation, however, some increases and adjustments are necessary to phase the new tumors into the program.

Some of the changes involved are as follows:

1. Techniques of using new tumors will have to be established and introduced into contractual screening operations.

2. In regard to animal production, the existing mouse production facilities can be converted to produce whatever new strains are needed, but the existing rat production is fully used and new facilities are being established in order not to hamper existing research programs. Good disease-free hamsters are virtually unobtainable and new production facilities are being acquired.

3. It is obviously impractical to screen all new materials introduced into the program against all available tumors. Procedures will be established for selective review of incoming compounds for analogues and homologues and directing them to selected screening systems.

Preliminary steps have already been taken in broadening the primary screen by adding two transplantable rat tumors (Walker 256 carcinoscarcoma and Dunning leukemia) to the standard three-tumor mouse screen (Sarcoma 180, Adenocarcinoma 755, Leukemia 1210). These two tumor systems are useful in predicting the effectiveness of anti-cancer agents against human leukemias and lymphomas, such as Hodgkin's disease. Tissue culture was added as a screening tool for the study of materials submitted in quantities insufficient for animal testing. Compounds showing activity in tissue culture can be synthesized in larger amounts for study in mice.

Secondary Evaluation and Methodology. Once a material has passed the primary screen, it is generally subjected to a broader exploration which is referred to as secondary evaluation. These experiments have several objectives:

1. To rank in order of interest closely related or new materials.

2. To test the breadth of activity of an agent or its ability to affect specific types of tumors.

3. To indicate the uniqueness of activity of the material.

4. To show the effects of agents on human tumors grown in a heterologous host, in order to assess the ability of the agents to affect human tumors of specific types.

In addition to animal testing, a very large part of the evaluation program involves testing and developmental research on the action of drugs in vitro. These investigations center largely around tissue culture and microbial studies. The progress in developmental research in these fields, particularly in tissue culture, has reduced the period of time required to fractionate and isolate an active component in crude antibiotic cultures from a period of years to a matter of only a few months.

The greatest part of the drug evaluation methodology effort of the program is directed toward a number of developmental approaches designed to explore new test systems which could ultimately replace or supplement the existing primary screen, develop methods for following up the fractionation and isolation of tumor-active agents in natural products such as antibiotic beers and extracts of the higher plants, and develop more information about materials which pass the primary screen both in animal tumors and in vitro.

A very important aspect of the preclinical plan of the CCNSC is the development and validation of "rating systems". These are animal tumor systems designed to tell which member of a series of homologues and analogues is most deserving of clinical trial. There are now available a reasonable number of well established "rating systems" and there are available a very large amount of data on the effectiveness of drugs in these various systems. However, one essential factor is lacking. It is not known how these drugs compare in all of the many responsive human tumors. Until correlation of the rating of these drugs in human disease with the animal disease ratings, it cannot be known for which human cancer the rating system is predicting. The validation of the predicting systems can only be secured with comprehensive data from a properly coordinated clinical program. Studies are being instituted to take full advantage of clinical experience data becoming available from the Center-sponsored clinical evaluation groups.

Toxicology and Pharmacology. The major objective of these studies to furnish the clinician with as much preclinical data about a drug as possible. Several contracts have been established to provide these data. Studies are made which enable the physician to evaluate new drugs in man without undue risks.

In the case of new agents, a relatively complete study is scheduled. Somewhat abbreviated studies are carried out with analogs of previously tested materials where toxicity experience has already been developed. In the case of steroids, which have generally low toxicity, or when the human dose has been projected, an abbreviated toxicology has also sufficed.

Endocrinology. A vast body of scientific fact and theory relate hormones to cancer. For this reason a search was organized for compounds with hormonal or anti-hormonal properties or the ability to alter the functioning of an endocrine gland. Many are worthy of intense study, since

small alterations of structure sometimes result in remarkable changes of physiological properties.

During the past three years approximately 900 steroids have been acquired for study. Of these, 193 have been recommended for clinical trial, and 69 are currently on trial. Trials on 25 materials have been completed, and two have been designated as sufficiently promising in breast cancer to warrant more extended trials. Some 400 steroids have been screened in the standard primary screen and more than 25 of these have passed one or more of the tumor tests. Consequently, consideration is being given to clinical trial of hormones in non-endocrine types of cancer, although at present the steroids are drugs of choice only in breast and prostatic cancer.

Clinical Trials. The cooperative clinical studies program (financed under Research Grant Projects) was organized to facilitate the clinical trial of agents developed through the preclinical testing program. The essential feature in this cooperative group enterprise is that the pooling of patients by several investigators shortens the time required to test a new material in any single type of malignancy. Additionally, a variety of approaches to the problem of drug evaluation in the cancer patient can be investigated, and the best methods instituted for the rapid trial of new agents. Important indirect benefits have resulted from the clinical cooperative program by placing greater emphases on the cancer patient; the quality of care for these patients has improved and data with regard to cancer complications and mortality have become available on a national basis.

Results of a clinical study sponsored by the Cancer Chemotherapy National Service Center indicated that women who received both radical surgery and chemotherapy for breast cancer were substantially less likely to have recurrent disease than were women treated only by surgery. In the study, 107 women, all of whom were considered good candidates for surgical cure, underwent radical mastectomy. Fifty-seven of the patients also received an anticancer drug, thio TEPA, administered intravenously on the day of operation and on the first and second post-operative days. The remaining 50 received a placebo. Data collected over a two-year period indicated that 83 percent of the women who received thio TEPA have remained free of recurrent disease, while only 54 percent of the other group were still free of cancer. Although the results of this study are preliminary, they constitute the first clear-cut evidence that adjuvant chemotherapy can markedly improve the benefits of surgery.

To date, 18 cooperative groups have been formed, and are composed of over 500 investigators in approximately 175 hospital services. Ninety-three studies have been completed, and 157 are in progress. Over 16,000 patients have been treated thus far.

A careful over-all analysis of the clinical program indicate that the emphasis of certain studies would implement the objective of obtaining an adequate trial of new types and classes of compounds for

treating cancer. Plans for the clinical program include two distinct types of clinical study which will receive emphasis in the future. These are:

1. Short term, preliminary trial of new agents in a broad spectrum of human malignancy. This is important in evaluation of new types of chemical structures, analogs of existing agents demonstrating greater activity in the laboratory, and those agents indicating a different mode of action. Important supplemental benefits will be gained from the establishment of possible relationships in different types of human tumors and the evaluation of the predicting ability of the animal tumor screening systems.

2. Quantitative, comparative studies in human malignancies responsive in some degree to chemotherapeutic agents to establish a ranking of relative therapeutic ability of agents. If comparative rankings are found in animal tumor "rating systems", this will aid immeasurably in the selection of the agent of choice for trial in specific human malignancy.

End Results Studies. A summary report on treatment and survival of more than 200,000 cancer patients was distributed at the Fourth National Cancer Conference, September 13-15, 1960. The report entitled "Survival Experience of Patients with Malignant Neoplasms", Public Health Service Publication No. 789, contains 115 pages of statistical data. In addition 8 analytical papers on end results of cancer therapy were presented to the various conference panels. These papers pertained to cancers of the breast, lung, female genital tract, gastro-intestinal tract, male genito-urinary tract, skin, head and neck, and hematopoietic system. A monograph analyzing trends in cancer survival rates and presenting detailed data on several relatively rare forms of cancer is in preparation.

Research Communications. Exchanges of technical information are helping accelerate chemotherapy research. To expedite communication in the field, the Cancer Chemotherapy National Service Center began publication of "Cancer Chemotherapy Abstracts", a monthly compilation of abstracts of reports appearing in journals throughout the world. A companion publication, now two years old, is "Cancer Chemotherapy Reports", which disseminates current information on the status of the program.

Program Plans

The over-all program will remain at approximately the same level. However, some shifts in emphases will occur as more new materials are found that are strikingly unique chemical structures in cancer chemotherapy, qualitatively different in modes of action in preclinical experiments, or analogs of compounds already of value displaying outstanding anti-tumor activity in a specific animal system as compared to the parent compound. These shifts would represent the focusing of interest on preparing and developing these materials for the clinic.

As these materials become increasingly available, the clinical program will be considerably augmented by transfers from other areas.

APPENDIX I

COOPERATIVE CLINICAL STUDIES

December 1960

Name of Group	Diseases Studied	No. Hospitals Participating
Acute Leukemia Group A	Acute Leukemia in Children	13
Acute Leukemia Group B	Acute Leukemia all Ages	12
Breast Group	Breast Carcinoma	32
Eastern Solid Tumor Group	Hodgkins Disease	8
	Pooled Lynphoma	
	Multiple Myeloma	
	Bronchogenic Carcinoma	
	Melanoma	
	Breast Carcinoma	
European Group	Breast	7
Midwest Group	Adult Acute Leukemia	18
	Variety of Tumors	
Prostate Cancer Study Group	Prostatic Carcinoma	11
Southeastern Group	Chronic Lymphocytic Leukemia	9
	Chronic Myelocytic Leukemia	
	Chronic Granulocytic Leukemia	
	Pooled Lymphoma	
	Multiple Myeloma	

Name of Group	Diseases Studied	No. Hospitals Participating
Southeastern Group cont.	Melanoma	
	Bronchogenic Carcinoma	
	Solid Tumors and Lymphomas	
	Breast Carcinoma	
Southwest Cancer Chemotherapy Study Group	Acute Leukemia in Children	13
	Childhood Solid Tumors	
	Chronic Lymphocytic Leukemia	
	Multiple Myeloma	
	Chronic Myelocytic Leukemia	
	Variety of Tumors	
Surgery Adjuvant Breast Group	Breast Carcinoma	25
Surgery Adjuvant Colon Group	Carcinoma of Colon	14
Surgery Adjuvant Lung Group	Carcinoma of Lung	23
Surgery Adjuvant Stomach Group	Carcinoma of Stomach	28
Surgery Adjuvant Ovary Group	Carcinoma of Ovary	24
Veterans Administration Chemotherapy Group	Carcinoma of Colon and Rectum	12
	Acute Leukemia Adults	

Name of Group	Diseases Studies	No. Hospitals Participating
Veterans Administration Chemotherapy Group cont.	Chronic Lymphocytic Leukemia	
	Hodgkins Disease	
	Chronic Granulocytic Leukemia	
	Pooled Lymphoma	
	Multiple Myeloma	
	Lung Carcinoma	
	Solid Tumors and Lymphomas	
	Melanoma	
	Carcinoma of Stomach	
Veterans Administration Surgery Adjuvant Group	Carcinoma of Colon	21
	Carcinoma of Lung	
	Carcinoma of Stomach	
Veterans Administration Lung (Hormone) Group	Bronchogenic Carcinoma	15
Veterans Administration Urological Group	Prostate	13
Veterans Administration Western Group	Leukemias	12
	Lymphomas	
Western Group	Chronic Lymphocytic Leukemia	5
	Chronic Myelocytic Leukemia	
	Hodgkins Disease	
	Solid Tumors	

Name of Group	Diseases Studied	No. Hospitals Participating
Western Group cont.	Variety of Tumors	
	Multiple Myeloma	

APPENDIX II

CCNSC CONTRACT OBLIGATIONS AS OF DECEMBER 15, 1960

Contractor	Cont. No.	Current Period	Purpose	Annual (or Current) Amount
Abbott Laboratories	3103	5/1/60-4/30/61	In-Plant Screen. and T. C. Methodol.	233,420
Alabama, Univ. of	3723	6/20/60-12/31/60	Histologic Studies of Lymphoma-Leukemia Complex	10,480
Arizona State University	1928	1/1/61-12/31/61	Synth. of Purine Derivatives	33,730
Arizona, Univ. of	3754	6/29/60 6/27/61	Development of Plant Extracts	19,800
Armour and Co.	1934	4/1/60-3/31/61	Preparation and Screen. of Animal Tissue Extracts	88,797
Armour Research Fdn.	1911	3/16/60-3/15/61	Primary Screening; Microbiol. Assays	264,979
Battelle Memorial Inst.	1913	5/1/60-4/30/61	Primary Screen.; Mouse Colony	324,975
Battelle Memorial Inst.	2369	7/1/60-6/30/61	Salmonella Diag. Lab.	23,767
Battelle Memorial Inst.	3012	6/23/60-1/31/61	Synth. of Free Radicals	19,080
Brandeis Univ.	3075	2/1/60-1/31/61	Biochem. of Alkylating Agents	26,670

Contractor	Cont. No.	Current Period	Purpose	Annual (or Current) Amount
Biologics Testing Lab.	3779	11/1/60-10/31/61	Toxicity Evaluations	66,040
Biologics Testing Lab.	3761	8/1/60-7/31/61	Endoc. Assays, Methodol., and Endoc. Tumor Devel. and Screening	420,142
Bristol Labs.	2411	1/1/60-12/31/60	In-Plant Screen. and Methodology	265,378
Bristol Labs.	2429	1/15/59-1/14/62	Production of Antibiotic	55,790
Bristol Labs.	3093	6/1/60-5/31/61	Development of Antibiotics; T.C. and Microbiol. Assay Methodology	570,396
Brooklyn Botanic Garden	2425	12/1/60-6/30/61	Human Tumor Screen.	28,148
Buffalo, Univ. of	3101	5/1/60-4/30/61	Experim. Design and Statist. Svcs.	23,885
Buffalo, Univ. of	3792	12/1/60-11/30/61	Development of Animal Tissue Extracts	39,000
Burrough Bros. Mfg. Co.	1950	12/1/60-11/30/61	Tableting	35,845
Burroughs, Wellcome and Co.,	3722	6/29/60-6/27/61	Prep. Lab. (Synthetics)	157,315

Contractor	Cont. No.	Current Period	Purpose	Annual (or Current) Amount
California Dept. of Public Health	3018	6/29/59-12/31/61	End Results Evaluation	15,989
California State Dept. of Public Health	2385	7/1/60-6/30/61	End Results Evaluation	41,619
California, Univ. of	2384	7/1/60-6/30/61	End Results Evaluation	18,834
California, Univ. of	3039	10/21/60-6/30/61	Clinical Data Proc. Systems	21,975
Carver Foundation (Tuskegee Institute)	2403	8/15/60-5/31/61	Tissue Culture Screen.	27,326
Charity Hospital of Louisiana	2393	7/11/60-6/30/61	End Results Evaluation	17,215
Charles River Breeding Labs., Inc.	3766	9/1/60-8/31/61	Fischer 344 Rat Supply	37,800
Chicago, Univ. of	1759	5/1/60-4/30/61	Steroid Synthesis	53,630
Chicago, Univ. of	2376	7/1/60-6/30/61	End Results Evaluation	15,371
Children's Cancer Res. Foundation	2421	11/1/60-10/31/61	Secondary Evaluations	140,352

Contractor	Cont. No.	Current Period	Purpose	Annual (or Current) Amount
Christ Hospital	1905	1/1/60-12/31/60	Eval. of Alkylating Agents	211,307
Cincinnati, Univ. of	3087	4/1/60-3/31/61	Preparation of Approx. 30 Compounds	2,354
Columbia Univ.	1917	7/1/60-6/30/61	Steroid Synth. and Bioassays	33,080
Columbia Univ.	2402	9/1/60-8/31/61	Endoc.-Related Tumor Development and Screen.	50,000
Cumberland View Farms	3765	10/16/60-10/15/61	BDF1 Mouse Supply	132,288
Cutter Laboratories	2438	5/1/60-4/30/61	Screening Methodology	61,951
Diablo Labs.	3796	12/15/60-12/14/61	Hybrid Mouse Supply	23,490
Documentation, Inc.	3794	12/1/60-4/30/61	Development of Data	491,042
Einstein Medical Center	2409	8/1/60-7/31/61	End Results Evaluation	10,707
Endocrine Consulting Labs.	3104	6/27/60-6/22/61	Steroid Bioassays	44,730

Contractor	Cont. No.	Current Period	Purpose	Annual (or Current) Amount
Endocrine Labs. of Madison, (Wisconsin)	2413	12/1/60-11/30/61	Steroid Bioassays	92,000
Georgetown University	2447	5/1/60-1/31/61	Chem. Synth.	10,999
Harvard Univ. School of Public Health (Mass. Dept. of Health)	2375	7/1/60-6/30/61	End Results Evaluation	25,942
Hazelton Labs., Inc. (Amendment and Renewal of FY 1958 Contract)	1912	6/1/60-5/31/61	Primary Screen. and Methodology	348,718
Hazleton Labs., Inc.	2360	6/1/60-5/31/61	Toxic Evaluations	140,609
Health Research, Inc. (Roswell Park Memorial Institute)	1876	7/1/60-6/30/61	Experim. Design for Clinical Studies	47,220
Health Research, Inc. (Roswell Park Memorial Institute)	2390	6/1/60-5/31/61	Iso-Antigenic Typing	20,000
Health Research, Inc. (Roswell Park Memorial Institute)	2415	10/1/60-9/30/61	End Results Evaluation	13,056
Hoffman-LaRoche, Inc.	3770	Delivery; 6/30/61	Production of 15 kgs. of 5-FUDR	187,500

Contractor	Cont. No.	Current Period	Purpose	Annual (or Current) Amount
Jackson, (R.B.) Memorial Lab.	3771	9/1/60-8/31/61	BDF1 and Inbred Mouse Supply	399,656
Jefferson Medical College	2387	1/1/61-12/31/61	Endocrine-Related Tumor Evals.	29,085
Kansas, Univ. of	2372	8/15/60-8/14/61	Mouse Colony	22,300
Lasdon Foundation	3056	2/1/60-3/31/61	Synth. of Steroids	56,400
Little, Arthur D., Inc.	1877	7/16/60-7/15/61	T.C. and Microbiol. Screen.; Methodol.	86,892
Little, Arthur D., Inc.	3789	1/1/61-12/31/61	Primary and Secondary Screens.; Pharmacol. and Methodol.	603,960
Lucille Scott Livingston Labs.	3032	5/1/60-4/30/61	Development and Screen. of Placental Lysates	60,000
Merck and Co., Inc.	1886	6/1/60-5/31/61	Biochem.; Histopathol; Methodol.	445,100
Merck and Co. Inc.	1948	2/1/60-1/31/61	Prep. Lab. (Steroids and Synthetics)	351,000
Merck and Co., Inc.	3057	6/1/60-5/31/61	Methodol: Eval. of Egg Host Screen.	138,000

Contractor	Cont. No.	Current Period	Purpose	Annual (or Current) Amount
Miami, Univ. of	1804	6/1/60-5/31/61	Tissue Culture Screen. and Methodol.	51,365
Miami, Univ. of	1956	4/1/60-3/31/61	Rat Leukemia Screen.	89,536
Miami, Univ. of	3040	9/1/60-8/31/61	Endocrine-Related Tumors: Development and Assays	98,840
Michigan, Univ. of	2374	7/1/60-6/30/61	End Results Evaluation	12,540
Michigan, Univ. of	3753	7/1/60-6/30/61	Experim. Design and Statist. Services	39,455
Michigan Dept. of Public Health	1921	7/1/60-6/30/61	Tissue Culture Methodol.	26,250
Microbiological Assocs., Inc.	2371	12/16/60-12/15/61	Secondary Evaluations	310,000
Microbiological Assocs., Inc.	2431	4/1/60-3/31/61	Primary Animal Screen.	520,572
Microbiological Assocs., Inc.	3076	1/15/60-1/14/61	Tissue Culture Methodol.	42,300
Midwest Research Inst.	3025	9/15/60-9/14/61	Synth. of Pyrimidines	250,750

Contractor	Cont. No.	Current Period	Purpose	Annual (or Current) Amount
Milan, Univ. of	3054	1/1/61-12/31/61	Comparative Secondary Screen. and Toxic. Evals.	27,360
Minnesota Mining and Mfg. Co.	3019	6/15/60-6/14/61	Synth. of Ethyleneimines	118,800
Monsanto Chemical Co.	3108	6/1/60-5/31/61	Synth. of P-Containing Antitumor Agents	84,300
New York Medical College	2377	7/1/60-6/30/61	End Results Evaluation	12,175
New York Univ. - Berg Institute	2391	7/1/60-6/30/61	Chronic Mouse Diseases Diag. Lab.	56,215
New York Univ. - Sch. of Med.	1904	3/1/60-2/28/61	Experim. Design; Stat. and Med. Mgmt. Svcs.	56,906
Norge Radiumhospital	3080	2/15/60-6/30/62	End Results Evaluation	39,640
North Carolina, Univ. of	1932	7/1/60-6/30/61	Experim. Design and Statist. Svcs.	25,970
Ochsner Fdn.	3036	7/1/60-6/30/61	Med. Mgmt.	15,638
Organon, Inc.	3091	6/1/60-5/31/61	Methodol. and Assays	65,200

Contractor	Cont. No.	Current Period	Purpose	Annual (or Current) Amount
Pabst Laboratories	2422	4/1/60-3/31/61	Synth. of Ribonucleotide Analogs	65,130
Parke, Davis and Co.	1965	12/1/60-3/31/61	Screening Methodol.	48,875
Pennsylvania, Univ. of	3058	12/1/60-11/30/61	Tissue Culture Methodol.	31,500
Pfizer, Charles, and Co., Inc.	1926	7/1/60-6/30/61	In-Plant Screen., Methodol., Pharmacnl., etc.	2,107,457
Philadelphia Ampoule Labs., Inc.	2426	11/15/60-11/14/61	Preparing Cmpnds. for Clinical Trials	59,360
Rawley Farms	3791	12/1/60-11/30/61	BDF1 Mouse Supply	106,080
Schering Corporation	1929	1/1/60-12/31/60	In-Plant Screen. and Methodol.	225,890
Schmidt, A. R., Co.	3800	1/1/61-12/31/61	BDF1 Mouse Supply	85,540
Scientific Literature, Inc.	3079	1/1/60-12/31/60	Scientific Abstracts	88,850
Scientific Literature Consults.	3107	6/8/60-3/7/61	Scientific Abstracts	58,380

Contractor	Cont. No.	Current Period	Purpose	Annual (or Current) Amount
Searle, G. D., and Co.	3061	6/1/60-5/31/61	Steroid Prep. Lab.	100,000
Seton Hall College	3016	7/1/60-6/30/61	Research on Lab. Animal Diseases	44,723
Simonsen Labs., Inc.	3776	11/1/60-6/30/61	BDF1 Mouse Supply	83,096
Sloan-Kettering Inst.	2445	5/1/60-4/30/61	Experim. Cancer Chemo.	1,826,002
Southern Res. Inst.	1740	7/1/60-6/30/61	Synth. of Purines	275,568
Southern Res. Inst.	2433	8/1/60-7/31/61	Biochem. Studies	155,676
Southern Res. Inst.	3784	12/1/60-9/30/61	Primary & Secondary Screens.; Methodology	858,014
South Shore Anal. and Research Labs.	2389	6/1/60-5/31/61	Toxic. Evaluations	94,325
Squibb, E. R. and Sons	2395	12/16/59-3/31/61	Endocrine Tumor Screen. Methodol.	209,009
Squibb, E. R. and Sons	3041	12/16/59-5/15/61	Steroid Prep. Lab.	706,619

Contractor	Cont. No.	Current Period	Purpose	Annual (or Current) Amount
Stanford Res. Inst.	1892	9/1/60-8/31/61	Synth. of Nucleosides	595,560
Stanford Res. Inst.	3068	12/15/60-11/30/61	Mech. of Action Studies	108,369
Stanford Res. Inst.	3070	12/1/59-3/31/61	Microbiol. Mech. Studies	100,339
Stanford Res. Inst.	3071	12/1/60-1/30/61	Primary and Secondary Screen.	307,104
Stanford Res. Inst.	3072	3/15/60-3/14/61	Secondary Evals. In Virus-T.C. Systems	88,596
Syntex, S.A.	2357	6/16/60-6/15/61	Steroid Prep. Lab.	75,220
Upjohn Co.	1856	12/1/60-11/30/61	Steroid Prep. Lab.	190,000
Upjohn Co.	1933	11/1/60-9/30/61	Drug Eval. and Develop.	422,813
Virginia, Univ. of	2379	7/1/60-6/30/61	End Results Eval.	11,712
Virginia, Univ. of	2388	9/1/60-8/31/61	Endocrine Tumor and Biochem. Studies	30,625

Contractor	Cont. No.	Current Period	Purpose	Annual (or Current) Amount
Wells Labs., Inc.	2401	7/1/60-6/30/61	Toxic. Evaluations Steroids	31,043
Wilson Labs.	2407	11/1/58 3/31/61	Prep. of Animal Tissue Extracts	34,000
Wisconsin Alumni Res. Fdn.	3083	1/1/60-12/31/60	Primary and Secondary Screen.	367,913
Wisconsin, Univ. of	2406	7/1/60-6/30/61	Screening Methodol.	64,959
Worcester Fdn. for Experimental Biology	3029	12/15/60-12/14/61	Development of Progest. and Anti-Progest. Assays	70,000
Wyeth Labs.	3073	3/15/60-3/14/61	Develop. of Antibiotics; In-Plant Screen.	552,909
TOTAL ACTIVE CONTRACTS				19,502,202

APPENDIX III

CCNSC RESEARCH CONTRACTS PROGRAM
ALLOCATION OF CONTRACT FUNDS

	FY 1961
DRUG DEVELOPMENT PROGRAM	$17,915,529
ACCESSORY PROGRAMS	2,352,506
END RESULTS PROGRAM	275,543
INFORMATION SERVICES	106,550
CLINICAL STUDIES *	729,552
GRAND TOTALS	$21,379,680

* Includes only those projects (e.g. Walter Reed, VA) supported by CCNSC contract funds.

CCNSC RESEARCH CONTRACTS PROGRAM
ALLOCATION OF CONTRACT FUNDS

DRUG DEVELOPMENT PROGRAM	FY 1961 Estimated Commitments			
	Totals	Sub-Totals	Amount	
I. Primary Screening	$12,101,229			
A. Compound Procurement		5,220,669		
1. Steroid Prep. Labs.			507,000	
2. Synthesis Contracts (non-steroids)			1,635,474	
3. Prep. Labs. for QNS			---	
4. Antibiotic Isolation			3,078,195	
a. Fungi				2,801,625
b. Higher Plants				167,570
c. Animal Products				109,000
B. Screening		6,880,560		
5. Animal Procurement			1,412,339	
6. Animal Disease and Tumor Control			165,495	
7. Animal Tumor Screening			3,368,071	
8. Tissue Culture			514,718	
9. Endocrine Testing			655,542	
10. Documentation			764,395	
II. Secondary Evaluation	2,900,042			
A. Procurement		378,171		
11. Prep. Labs.			278,171	
12. Purchases			100,000	
B. 13. Tumor Systems		1,715,315		
C. 14. Microbiological and Tissue Culture		422,356		
D. 15. Biochemical		384,200		
III. Preparation for Clinical Trial	2,914,258			
A. Procurement		1,582,636		
16. Prep. Labs.			1,000,396	
17. Purchases			582,240	
B. 18. Pharmacology		816,405		
C. 19. Dosage Form Production		122,267		

ALLOCATION OF CONTRACT FUNDS

	FY 1961 Estimated Commitments		
	Totals	Sub-Totals	Amount
D. 20. Control Labs.		61,500	
E. 21. Clinical Mgmt. and Statistical Services		331,450	
Totals for Drug Development Program	$17,915,529		
ACCESSORY PROGRAMS			
I. (22) Screening Methodology	$ 814,492		
II. (23) Endocrinology: Research and Methodology	799,980		
III. (24) Biochemistry and Biology	738,034		
Total Accessory Programs	$ 2,352,506		
End Results Program	$ 275,543		
Information Services	$ 106,550		

APPENDIX IV

TRANSFERS OF FUNDS TO

OTHER FEDERAL GOVERNMENT AGENCIES

Agency	Purpose	FY 1961 Amount
Agriculture, Department of	Collection of Plant Extracts	$ 45,000
Army Chemical Center Department of the Army, DD	Synthesis of Nitrogen Mustards	25,000
Food and Drug Administration DHEW	Toxicity Evaluations	25,000
Veterans Administration	Clinical Studies	768,000
Walter Reed General Hospital WRAMC	Toxicity Evaluations	26,552
TOTALS		$889,552

Serial No. NCI-193
1. Associate Director for Field Studies
2. Nursing Section
3. Bethesda, Maryland

[illegible]-NIH
Ind[illegible] Project Report
[illegible] Year 1960

Part A

Project Title: Cancer Nursing Bibliography - Supplement

Principal Investigator: Patricia B. Geiser

Other Investigators: Elizabeth Walker

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1/4
Professional: 1/8
Other: 1/8

Project Description: Annotated bibliography on articles published in 1959 and early 1960

Part B included Yes [x] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publication: Mimeographed "Supplement" to the published "Bibliography on Cancer for Nurses"

U. S. Department of Health, Education, and Welfare, Public Health Service, National Cancer Institute, October 1959, PHS Publication 687

Serial No. NCI-194
1. Associate Director for Field Studies
2. Nursing Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Preparation of a Monograph on Radiation (For Nurses)

Principal Investigator: Elizabeth Walker

Other Investigators: Rosalie I. Peterson

Cooperating Units: Advisory Committee comprised of Representation From:

1. U. S. Army
2. U. S. Navy
3. Veterans Administration
4. Brookhaven National Laboratory
5. Oak Ridge National Laboratory
6. University of Pennsylvania
7. Roswell Park Memorial Hospital

Man Years (calendar year 1960):
Total: 3/4
Professional: 1/2
Other: 1/4

Project Description:

Objectives:

1. To make available, in one reference, the information which a nurse needs to function effectively in providing care to patients receiving X-ray, radium or radioisotope therapy.

2. To explain pertinent basic information about radiation in a simple and direct way.

3. To make provision in this publication for yearly supplements or replacements as needed.

Methods Employed:

An advisory committee (as noted above) is working with the principal investigators to help determine what information is most needed and how this can best be presented. This group is consulting with medical (particularly radiologists) and nursing staffs in each institution represented. It is planned that written materials will be submitted to the group by those having special interest in certain areas; these will be reviewed and incorporated in the manual by the principal investigator. Illustrative material from the various centers is also to be submitted for review and inclusion as deemed advisable.

Significance:

There is unanimous feeling among the advisory group that there is a definite need for such a publication and that it should be made available as soon as possible. Several of the physicians associated with the nurses on the committee have also expressed their interest in seeing this publication completed in the near future.

Proposed Course:

During 1960 the Advisory Committee continued to function but with a loss of membership. Major Hays of Walter Reed was assigned to Korea, but will continue to serve in absentia. Miss Best of M. D. Anderson Hospital resigned her position and moved to another state.

In 1960 several drafts of the manual were submitted for review, but much still remains to be done. Since the committee is comprised of people with full time positions, progress is necessarily slow. It is hoped that the next few months will see the completion of this project, since there is great demand from the field for this publication. Consultation is being provided from the Robert A. Taft Engineering Center in Cincinnati, Ohio, and the efforts of our group are being coordinated with other governmental groups responsible for the radiological education of nurses.

Part B included Yes ☐ No ☒

Serial No. NCI-195
1. Associate Director for Field Studies
2. Nursing Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Investigation of Problems (Mental and Physical) Faced by Patients Undergoing Treatment for Cancer at Roswell Park Memorial Institute

Principal Investigator: Earlene T. Finnerty

Other Investigators: Rosalie I. Peterson and Elizabeth Walker

Cooperating Units: None

Man Years (calendar year 1960):
Total: 4/4
Professional: 3/4
Other: 1/4

Project Description:

A pilot study to test the feasibility of studying post-hospitalized cancer patients for a specified period of time in order to find out some of the major problems faced by the patient and his family.

Objectives:

1. To learn more about the fears and anxieties of cancer patients and the problems which may delay or detract from the fullest rehabilitation of these patients.

2. To ascertain the extent to which nursing service may help meet such problems.

Methods Employed:

Data was collected on colostomy patients through unstructured interviews which were tape recorded. Interviews were conducted in patients' homes or in the outpatient clinic.

Significance to Cancer Nursing:

It is felt that a study of this kind will point up certain information which may help nursing leaders to prepare student nurses to meet the problems of individual cancer patients and to provide support to the patient at the time it is most needed.

Proposed Course:

The first portion of the study was delimited to patients who had had colostomies. The next step in the study will be the development of a structured interview form developed from data secured from unstructured interviews. The structured interview will be tested for validity and the refined form will be used in several agencies or institutions in various geographic areas for the collection of data.

Part B included Yes ☐ No ☒

Serial No. NCI-196
1. Associate Director for Field Studies
2. Nursing Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Consultation to Agencies and Universities Interested in Specialized Programs in Cancer Nursing (Baccalaureate and Graduate programs)

Principal Investigators: Rosalie I. Peterson and Elizabeth Walker

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1/2
Professional: 1/2
Other: 0

Project Description:

Several visits have been made to New York University, Teachers College, Columbia University and other universities, to explore the need for courses which offer nurses the opportunity for special preparation in cancer nursing in programs leading to master's and doctoral degrees. Consultation has also been carried on in several basic programs, i. e., on a baccalaureate level, in such schools as the University of Washington, University of Oregon and University of California.

Proposed Course:

To continue consultation as solicited and to further interest in expanding the clinical programs in Cancer Nursing.

Part B included Yes ☐ No ☒

Serial No. NCI-197
1. Associate Director for Field Studies
2. Nursing Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Film on Cancer Nursing

Principal Investigator: Rosalie I. Peterson

Other Investigators: Elizabeth Walker

Cooperating Units: American Cancer Society

Man Years: (calendar year 1960):
Total: 1/4
Professional: 1/4
Other: 0

Project Description:

Objective: To produce a film on nursing care of patients with cancer--one designed specifically for a nursing audience.

Significance: Through this film we hope to promote better understanding of both the psychological and physical problems of patients with cancer.

Part B included Yes ☐ No ☒

Serial No. NCI-198
1. Associate Director for Field Studies
2. Nursing Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: A pilot study to determine if there might be enough life experience available among the female nurse officers of the Commissioned Corps (1944-1959, inclusive) to study the incidence of cancer and/or other diseases among these officers..

Principal Investigator: Patricia B. Geiser

Other Investigator: Dr. Alexander G. Gilliam

Cooperating Units: None

Man Years (calendar year 1960):
Total: 3/4
Professional: 1/2
Other: 1/4

Project Description:

Objectives:

To see if there is sufficient life experience represented among female commissioned nurses to determine if their cancer risk is greater or less than for women in the general population.

Methods Employed:

1. Employ the actuarial method of calculating the expected incidence of cancer and other diseases in a selected population group.

2. Determine the actual incidence of these diseases in this population.

3. Determine the significance of a comparison of these findings.

Results:

1,004 female commissioned nurses had active duty experience, 1944-1959, inclusive. 635 of these had their active commissions terminated, 55 retired for disability, 10 for age and 5 through death. In view of the fact that development and diagnosis of a disabling condition resulted in disability retirement, no direct comparison between the 22 deaths expected, and 5 observed, is valid. For a more valid comparison, one must add the life experience of the 62 retirees; compute and add the fractional expected deaths in that life experience; and add to observed deaths those which have occurred among these 62 retired. The basic information for this is available in D.C.O., but was not available on the qualification records used for the study.

Proposed Course of Action:

No further study is anticipated.

Part B included Yes ☐ No ☒

BIOMETRY BRANCH
Office of the Associate Director for Field Studies
National Cancer Institute

ANNUAL REPORT FOR 1960

The activities of the Biometry Branch during 1960 continued along the lines of the master plan described in the Summary Report for 1954-1959. In addition to research in the methodological aspects of mathematical statistics and in substantive investigations of the distribution of cancer risk among population groups, utilizing cancer register, morbidity survey, and vital statistics sources, the Branch carried on the following activities: (a) Collaboration and assistance in statistical problems arising in laboratory and clinical studies; (b) continued support and development of the statistical staff and program of the Cancer Chemotherapy National Service Center; (c) direction of the End Results program as a collaborative venture with the CCNSC; (d) collaboration and assistance in projects in geographic pathology undertaken by the Laboratory of Pathology; (e) assignment of key personnel and support of the statistical needs of the Epidemiology and Diagnostic Research Branches and other activities within the Field Studies Area. The Branch has maintained a flexible approach to these activities and considers each need on its own merits. Where indicated the Branch may retain the technical function and activity; other needs require the establishment of statistical units in other Branches and the maintenance of consultative relationships only.

Work with agencies inside and outside the Public Health Service expanded. The Branch consulted and cooperated with the National Office of Vital Statistics, the Cancer Control Branch of the Bureau of State Services, the American Cancer Society, and the Sloan-Kettering Institute. Our consultant relationship with the Joint Committee (American Colleges of Surgeons, Physicians, Radiologists, and Pathologists) on Staging and End Results Reporting deserves special mention. The problem of uniform definitions and staging is a pressing one in the program of the Clinical Biometry Section and an essential ingredient for achieving comparable survival rates for cancer treatment. The Joint Committee has made substantial strides in specifying detailed criteria for the staging of several sites and the Branch is in a position to make an important contribution by providing facilities for testing the definitions in field trials.

The expansion of NCI Field Study activities, symbolized by the appointment of an Associate Director for Field Studies, creates new problems and new opportunities. During the past few years the greatest growth in statistical consultation has been in connection with studies involving collaboration with other organizations, particularly those stimulated by CCNSC. The new and expanded Field Study activities will involve intimate collaboration with groups of investigators outside NCI, and statistical consultation commitments to such programs will

increase, especially in the areas of diagnostic test research, epidemiology, and environmental carcinogenesis. Plans to support work in research centers in Connecticut and California based upon population laboratory concepts would also add further pressures to the load carried by the Branch.

The statistical stakes in the collaborative enterprises have become so great that there is a need to designate a senior statistician to handle the broad statistical planning and policy aspects of these studies. Failure to provide the proper support and to maintain the highest standards in statistical analysis could possibly lead to failure or collapse of these studies. The Associate Branch Chief, Mr. Schneiderman, has been asked to assume direct responsibility for coordinating statistical programs in the collaborative study area.

The trend of events makes it increasingly clear that the statistical needs of the National Cancer Institute cannot be met by a highly centralized statistical organization. Further decentralization with an increasing number of statistical units assigned to other program activities is indicated. This will involve considerable work over the next few years in defining operational needs, matching statisticians, with the appropriate talents, with the positions to be created, and recruiting many additional statisticians capable of filling research positions. The Chief, Associate Chief, and Section Heads will give high priority to these needs.

The volume of study activities makes our data processing needs more acute. The solution lies in more intensive use of computer facilities and other mechanical aids for data processing. The NIH computer organization appears unable to take care of all our programming needs and it may prove advantageous for the Branch to have at least one programmer on its staff. This might lead ultimately to the creation of a section to handle computer programming which could then engage in its own program of methodological research to devise new solutions to the data processing problems confronting us.

During the past year the Branch continued its general program of activities with substantially the same personnel and approximately the same budget that it has had for the past several years. Four developments are singled out for comment:

(1) End Results Program: The highest priority was given to the preparation of End Results data at the 4th National Cancer Conference, derived from more than 200,000 diagnosed cases in three population-based cancer registers and seven individual hospital registers. The End-Results presentations were made an integral part of several panel discussions organized around specific sites at the Minneapolis Conference. This work was the natural result of the long-term commitments with the Connecticut Cancer Register and our pioneer work in organizing the

End Results Group following the program on End Results in Cancer at the 3rd National Cancer Conference. A summary volume, entitled "Survival Experience of Patients with Malignant Neoplasms", was distributed at the Conference; staff members collaborated with the designated End-Results reporters in the preparation of papers to be published in the Conference Proceedings.

Much of these data will be presented and analyzed in greater detail in a monograph to be ready for publication early in 1961. A substantial amount of planning went into the preparation of the monograph format and the intent is to have a cyclical presentation scheme concentrating on a few sites in detail each year. Two special studies dealing with specific problems of cancer treatment are underway. Both were suggested by Dr. Shimkin and involve the analysis of data supplied by selected institutions. One (with the cooperation of Dr. Ochsner and Dr. Overholt) concerns a comparison of lobectomy versus pneumonectomy in the treatment of lung cancer; the other deals with an analysis of results of simple and radical mastectomy in breast cancer arising from a unique study situation in Rockford, Illinois.

A series of publications on the long-term survival of cancer patients based on the experience of the Connecticut Register continued during 1960, with papers reporting on stomach, large intestine and rectum, appearing in the Journal of the National Cancer Institute.

(2) International Program: The collaborative program in end results and epidemiology of cancer now underway between the United States and Denmark, England, Finland, France, and Norway was further implemented by the negotiation of contracts with Cancer Registries in the three Scandinavian countries. The contracts call for the delivery of punch cards prepared in a uniform manner based on definitions and criteria agreed to by all collaborating parties. These data will be processed, using the NIH computer program, and will contribute greatly to an analysis of the international variation in end results and lay the groundwork for the design of more sophisticated studies at a later date. Much of these data will be presented at the 8th International Cancer Congress in 1962.

A pathological review of cervical cancer cases in Connecticut and in England by a team of two pathologists, Dr. Thomas of the National Cancer Institute and Dr. Thomson of the Bland-Sutton Institute of Pathology, was completed in September 1960. The review had been suggested by some very striking differences in five-year survival rates reported from England and the United States. The material is now being analyzed and a series of papers is projected. Apart from the contribution made to the substantive issues involved, the project can be considered as a prototype of how pathologists can use cancer register data as a base for more detailed investigations of geographic pathology. At the urging of Dr. Stewart and Mr. Haenszel, the

reviewing pathologists and others concerned have agreed to prepare an expository paper devoted to the methodological aspects of the study.

During the past year, exploratory conversations were carried on with epidemiologists in Japan concerning possible studies on shifts in cancer risk among Japanese living in Japan, Hawaii, and the continental United States. The mortality data now available suggest that there are distinct differences in risk for specific sites. These interests will be pursued in 1961, and we look forward to the development of a study plan for one or two sites.

The Branch interest in international comparisons and studies of gradients in cancer risks for migrant groups has been greatly stimulated by the findings on cancer mortality among the foreign-born in the United States. A paper on this subject will appear in the January 1961 issue of the Journal of the National Cancer Institute. These data exhibit quite specific configurations of risk by site and ethnic group. For some sites there is evidence of a substantial carry-over in risk by migrants from country of origin to country of destination. The United States is the destination of one of the great population movements of modern times, and the presence of so many migrant groups places us in an especially favorable position to plan and conduct studies of effects which may be linked to changes of environment.

(3) Intensive Use of Mortality Data: The Branch has experimented with a dual sampling of deaths and the corresponding population at risk to elaborate information available from conventional vital statistics sources. The pioneer study of a 10% sample of lung cancer deaths in the United States for 1958, involving the collection of detailed residence, smoking, and occupation histories, is nearing completion and a paper reporting the results for white males is being readied for publication. The major finding is the trivial urban-rural difference in risk for non-smokers which persists for all criteria used to measure urban and rural residence. There is a definite interaction between smoking history and residence which is expressed by a very large urban-rural difference in risk for heavy smokers (persons using more than one pack of cigarettes daily). A corresponding paper reporting on white females, for whom data collection was extended for another year - through 1959, will appear later.

The conduct of the study demonstrated that this method has obvious applicability for investigations of other sites, and represents a quick economical approach for securing information on distribution of risk, which can be utilized for planning more expensive and elaborate longitudinal studies.

(4) Methodological Studies: During his tenure as a Rockefeller Public Service Fellow, Mr. Schneiderman investigated the properties of various sequential sample schemes and developed some new approaches. His findings, which are being reported in a series of publications, have obvious implications to the design of therapeutic trials conducted in connection with the Chemotherapy Program.

Mr. Cutler, in his Doctoral dissertation, which has been accepted by the University of Pittsburgh, reviewed intensively the use of interval survival ratios as a tool for analyzing end-results data. His work indicates that the shape of survival curves differs by site and that curve-fitting procedures developed by Boag and others, which do not allow for site-specific differences, may yield misleading results. The analysis of end results data undertaken within the Biometry Branch will continue to lay stress on the specific configurations of interval survival ratios.

Serial No. NCI-50
1. Biometry Branch
2. Office of the Chief
3. Silver Spring, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Activities during 1960

Principal Investigator: W. Haenszel and M. A. Schneiderman

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: 3½
Professional: 1½
Other: 2

Project Description: The activities of the Office of the Chief have included the following:

(1) General planning and direction of the scientific program of the Branch, including recruitment and development of statistical staff and coordination of activities of statistical units assigned to other branches of the National Cancer Institute.

(2) Development of international cooperation of tumor registries. During 1960 contracts were negotiated with the Cancer Registries in Denmark, Finland, and Norway, calling for the preparation of cancer end-results data in a form comparable to that prepared by American Registries. To investigate the great difference in survival experience between cervical cancer patients in England and the United States, a plan was developed in cooperation with the Laboratory of Pathology, NCI, for a pathological review of a series of specimens drawn from representative cancer registries in each country. The Branch negotiated the study arrangements and gave staff support to the team of two reviewing pathologists.

Exploratory talks were held with Japanese epidemiologists concerning collaboration in studies to investigate the gradients in cancer risk for specific sites among Japanese in Japan, Hawaii, and the continental United States. Agreement in principle has been reached on such studies and further meetings will be held in 1961 to implement decisions on specific study plans.

(3) Participation in development of study plans and activities of closely related organizations; member of American Cancer Society's Standing Committee on Statistics and Ad Hoc Committee on Epidemiology (Mr. Haenszel); consultant, Joint Committee on Staging and End Results Reporting sponsored by the American Colleges of Surgeons, Physicians, Radiologists, and Pathologists (Mr. Haenszel); member, National Research Council's Sub-committee on Hematologic Effects of Atomic Radiation (Mr. Schneiderman).

(4) Consultation on cancer epidemiology and cancer control to Cancer Control Branch, BSS.

(5) Participation in activities of Committee on Geographical Pathology of the International Union Against Cancer, including preparation of a paper read at its October 1960 Symposium in Tokyo.

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:
Haenszel, W.: Mortality and Morbidity Statistics on All Forms of Cancer. To be published in Acta Unio Contra Cantrum.

Honors and Awards relating to this project:
Mr. Schneiderman has been elected a member of the Council of the Biometric Society.

Serial No. NCI-52
1. Biometry Branch
2. Demography Section
3. Silver Spring, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Cancer Morbidity Surveys in the United States

Principal Investigator: William Haenszel

Other Investigator: Leonard Chiazze

Cooperating Units: None

Man Years (calendar year 1960):
Total : 0
Professional: 0
Other : 0

Project Description:

(A) Objectives: To provide basic data on cancer incidence, prevalence, mortality, and survival experience in the United States; investigate effect of various demographic factors, such as race, sex, age, geographic region and economic status.

(B) Methods employed: Information on cancer patients seen during a specified calendar year is obtained from virtually all diagnostic sources (hospital, clinic, laboratory, physicians) in each area surveyed. Lists of diagnosed cases are subsequently searched against death indexes in vital statistics offices. Hospitals and physicians are also contacted for further information on patient status.

(C) Major findings: Some of the earlier findings on cancer incidence from the ten-city morbidity surveys have been published in Public Health Monograph No. 29, Morbidity from Cancer in the United States. The second monograph in this series published at the end of 1958, discussed intercity variation in cancer incidence, relationship of cancer incidence to economic status, changes in cancer morbidity between 1937 and 1947, age distribution of cases diagnosed at different stages and a review of the consistency of cancer incidence data from various sources. Cancer Morbidity in Urban and Rural Iowa appeared as Public Health Monograph No. 37.

Project Description: (cont.)

In three cities (Dallas, Chicago, Pittsburgh) and the State of Iowa, the diagnosed cases enumerated in the morbidity surveys have been traced to obtain survival information for a five-year period. Searches against death indexes in vital statistics offices have been completed in the four areas, and this data has been coded and punched for use in IBM tabulations.

(D) Significance to the program of the Institute: Provides basic epidemiological information regarding the incidence of cancer.

The survival experience of cancer patients enumerated in morbidity surveys will prove useful in resolving questions raised about the comparability of data secured by case register and morbidity survey techniques.

(E) Proposed course of project: All the coding and record processing in preparation for a monograph analysis of the five-year survival rates for diagnosed cases in the morbidity surveys has been completed. The IBM-650 Computer Program for processing the survival data has been available for several months. The computer time has been preempted for preparing data for the End Results Group presentation at the 4th National Cancer Conference hence, this particular work had to be set aside. Computer processing is now under way and will be completed early in 1961; analysis of the data and preparation of a paper will follow.

Part B included Yes [ ] No [X]

Serial No. NCI-53
1. Biometry Branch
2. Demography Section
3. Silver Spring, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Statistical Studies of Cancer in Pittsburgh Area

Principal Investigators: William Haenszel and Antonio Ciocco

Other Investigators: Sidney J. Cutler, Dorothy Perrott, and Leonard Chiazze

Cooperating Units: University of Pittsburgh School of Public Health

Man Years (calendar year 1960):
Total: 2
Professional: 1 1/2
Other: 1/2

Project Description:

(A) Objectives: To pursue a series of intensive cancer morbidity studies in a single geographic area to obtain information on factors contributing to unusual incidence for specified sites. The collation of data obtained by a variety of approaches to the same population base will permit stronger inferences to be drawn from the chain of evidence, open the way to designing detailed studies to test specific hypotheses suggested by the evidence, and make possible methodological contributions to field studies of chronic illness in human populations.

(B) Methods Employed: A cancer registry, which receives reports from virtually all hospitals in Allegheny County, is being operated in cooperation with the State and County Health Departments, and the School of Public Health. The information received from the hospitals was supplemented in 1959 by a canvass of private physicians' records pertaining to cancer patients seen in 1957 and 1958. During the past year, the data obtained from private physicians has been collated with the hospital reports and listings of cancer deaths obtained from the Bureau of Vital Statistics. The collected material is being coded and entered on punch cards. The initial analytical phase of the project will deal primarily with the following questions: (1) What proportion of all cancer cases identified

Part A - continued

by tapping a variety of sources of information were reported by the hospitals participating in the continuing cancer registry program; how many additional cases were obtained by tapping each of the various other sources of information? (2) What changes in cancer incidence rates occurred since the previous morbidity survey in 1948? (3) What is the geographic distribution of the various forms of cancer within the county; what are the demographic characteristics of persons living in high incidence areas, in low incidence areas?

(C) Major Findings: Collected material is now being coded and entered on punch cards.

(D) Significance to the Program of the Institute: The basic notion of epidemiological studies of a population in depth has never been attempted on this scale for cancer. As mentioned in the objectives, these studies will contribute valuable information on methodology in field studies of chronic illness in human populations.

(E) Proposed Course of Project: The analyses of morbidity data by census tracts will supply leads for the design of studies to test specific hypotheses, which would open the second phase of the project. Such studies would utilize the unique sources of information available locally, such as: the "population laboratory" in the Arsenal Health District and 1% city sample, and the resources of the Department of Occupational Health (in the School of Public Health) in gaining access to records of people working in plants in the Pittsburgh area.

Part B included Yes ☐ No ☒

Serial No. NCI-55
1. Biometry Branch
2. Demography Section
3. Silver Spring, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Retrospective Study on the Association of Smoking and Lung Cancer In Women

Principal Investigators: William Haenszel and Michael B. Shimkin

Cooperating Units: 63 institutions throughout the United States

Man Years (calendar year 1960):
Total: 0
Professional: 0
Other: 0

Project Description:

(A) Objectives: To observe whether the association between smoking and lung cancer observed repeatedly for males holds also for females. A study among women will permit estimates of the separate effects of chronological age and duration of smoking, since women of all ages took up smoking in the 1930 decade.

(B) Methods Employed: Histories of smoking and other characteristics are obtained for each diagnosed lung cancer case and two matched controls. The cooperation of 63 institutions has been secured and histories are being collected by persons connected with each institution. The small number of lung cancer cases among women make cooperative study arrangements necessary.

(C) Major Findings: Histories have now been received for 302 women lung cancer cases and 604 matched controls, much larger than any other series reported in the literature. A paper reporting the results for the first 192 cases has been published. The findings are consistent with other studies on women reported in the literature in indicating that the excess risk among female smokers is concentrated in the epidermoid and undifferentiated types. Also, the combined results of several investigations suggest that the characteristic excess lung cancer mortality among males almost disappears when nonsmokers are studied, since male nonsmokers have only slightly higher rates than female nonsmokers.

Part A - continued

(D) Significance to the Program of the Institute: See (A)

(E) Proposed Course of Project: Collection of histories has been discontinued; analysis of the more extended series has yielded findings which agree substantially with those derived from examination of the first 192 cases (See - Haenszel, W. and Shimkin, M. B.: Lung Cancer Among Women. Acta Unio Internat. Cantra Cancrum, 15: 493-501, 1959). Since no new information nor data modifying the earlier conclusions have resulted, no further publications are contemplated and this project is terminated.

Part B included Yes ☐ No ☒

Serial No. NCI-56
1. Biometry Branch
2. Demography Section
3. Silver Spring, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Association of Smoking History, Residence History, and Occupation History With Lung Cancer Mortality

Principal Investigators: William Haenszel and M. G. Sirkan (National Office of Vital Statistics)

Other Investigators: D. Loveland and Karl Taeuber

Cooperating Units: National Office of Vital Statistics

Man Years (calendar year 1960):
Total: 3 1/2
Professional: 2
Other: 1 1/2

Project Description: This project is being undertaken in collaboration with the National Office of Vital Statistics, Division of General Health Services, Bureau of State Services.

(A) Objectives: To study the distribution of lung cancer mortality among population sub-groups classified with respect to smoking history, residence history, and work history. The interaction of these three variables in influencing lung cancer mortality rates is also being investigated.

The residence history information collected for a sample of the general population is also being analyzed separately in its own right as a unique source of data on migration. The interpretation of cancer incidence data is at times complicated by changes of residence of ill people for reasons of health. Information on population movements of the general population will be most useful for investigations of this subject.

(B) Methods Employed: Detailed smoking, residence, and work histories have been collected for samples of deaths from relatives of the decedent and the attending physician. Additional diagnostic information on which the medical certification of death was based is also gathered. Most of the data have been collected by mail

Project Description (cont.)

(B) Methods Employed (cont.)

questionnaires. Corresponding data on smoking, residence, and work histories for the general population were collected under contract with the U. S. Bureau of the Census as a supplement to their Current Population Survey.

Collection of data for deaths and the population base in this manner permits extension of the range of variables for which mortality rates can be computed.

(C) Major Findings: The methodological problems of studies involving collection of dual samples of decedents and of the general population at risk have been investigated and successfully surmounted. The conduct of a pre-test on a sample of 1800 deaths in the State of Pennsylvania yielded as a by-product considerable descriptive data dealing with the diagnostic evidence supporting medical certifications of death. The analysis of the histories for 2600 male lung cancer deaths in the national 10% Current Mortality Sample for 1958 is nearing completion. The outstanding finding to date is the trivial urban-rural difference in lung cancer mortality among non-smokers. The urban-rural differential increases with the amount smoked and is largest for smokers regularly using more than one pack of cigarettes daily. This finding of an interaction between smoking and residence history persists for all residence classifications attempted, including those based on usual residence at time of death and those derived from a lifetime history.

(D) Significance to the program of the Institute. There are several unresolved issues in the epidemiology of lung cancer. Several of these are concerned with the interaction among the effects of smoking history, atmospheric pollution, and specific work exposures in influencing excess lung cancer risks. Studies of lung cancer mortality such as that discussed here will provide useful information for the planning of forward studies in urban areas thought to have severe atmospheric pollution problems.

(E) Proposed course of project: A paper reporting the preliminary results for the 10% sample of male lung cancer deaths was presented at the New York Academy of Medicine Symposium on Smoking and Health (September, 1960). A more detailed paper is being readied for publication and will be completed early in 1961. This will be followed by a paper reporting parallel data for approximately 800 female lung cancer deaths. The latter will pay particular attention to the sex difference in urban-rural ratios of lung cancer risk and the hypotheses advanced in the

Project Description (cont.)

(E) Proposed Course of Project (cont.)

literature to explain this phenomenon. Additional data on samples of deaths from cancer of the large intestine and rectum, originally collected as controls for the lung cancer deaths, will be analyzed separately in their own right as a third paper.

A monograph containing a systematic analysis of migration patterns based on the residence histories collected by the Bureau of the Census in its Current Population Survey is planned. This is in addition to a series of papers reporting on the migration data and relation of them to current sociologic and demographic theories and hypotheses.

Part B included Yes [x] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

(1) Sirken, M. G., Haenszel, W., and Pifer, J. W.: Residence Histories of Deceased Persons. Milbank Memorial Fund Quarterly, 30: 5-22, 1960.

(2) Haenszel, W.: Quantitative Evaluation of the Etiologic Factors in Lung Cancer. Presented at the New York Academy of Medicine Symposium on Smoking and Health. Sept. 26-27, 1960. (To be published in the Proceedings of the New York Academy of Medicine.)

(3) Taeuber, K. E.: Duration-of-residence Analysis of Internal Migration in the United States. (To be published in the Jan. 1961 issue of the Milbank Memorial Fund Quarterly.)

(4) Sirken, M. G., Pifer, J. W., and Brown, M. L.: Survey Procedures for Supplementing Mortality Statistics. Am. Jr. Public Health 50: 1753-1764, 1960.

Serial No. NCI-57
1. Biometry Branch
2. Demography Section
3. Silver Spring, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Studies of Cancer Mortality Data

Principal Investigators: William Haenszel and Tavia Gordon

Other Investigators: Margaret Crittenden

Cooperating Units: None

Man Years (calendar year 1960):
Total: 2
Professional: 1
Other: 1

Project Description:

(A) Objectives: To investigate possible applications of mortality data for epidemiological study purposes.

(B) Methods Employed: Special tabulations of cancer mortality have been secured from the National Office of Vital Statistics and a series of charts on regional variation in cancer mortality by site within the United States is being assembled. A duplicate set of punch cards for 1950 cancer deaths has also been obtained and the microfilms of death certificates have been reviewed to add country of birth of decedent to the punch cards. Tabulations of cancer mortality by occupation have also been obtained from the National Office of Vital Statistics.

(C) Major Findings: There are significant regional differences in cancer mortality by primary site within the United States. Mortality trends are also different for specific primary sites. The available evidence from cancer register and morbidity surveys in general support the inferences to be drawn from the mortality data.

Striking differences in cancer mortality by site have been found among the foreign-born when classified according to country of origin. The sites which exhibit the greatest differences among ethnic groups and between native whites and foreign-born whites

Part A - continued

include esophagus and stomach. The range of differences are much smaller for sites in the lower digestive tract (large intestine and rectum) and such other sites as bladder and leukemia and the lymphomas.

The systematic analysis of cancer mortality among the foreign-born groups in the United States is scheduled for publication. A number of study plans are being developed to exploit these findings and will emphasize the study of cancer risks among native whites of foreign parentage and collaborative studies with investigators in such countries as Norway, England, and Japan to trace gradients in cancer risk between populations in the country of origin and country of destination.

(D) Significance to the program of the Institute: The mortality data thus obtained will be useful in furnishing leads and deliniting subject matter areas for investigation by other study techniques. These studies provide essential baselines for cancer investigations.

(E) Proposed course of project: The systematic review of trends in morbidity and mortality data by cancer site for the period 1930-1955 is near completion and should be ready for publication in the first quarter of 1961. The intention is to follow this up from time to time with systematic reviews for specific cancer sites, the next site for which a review is contemplated being esophagus.

Part B included Yes [x] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

PART B. Honors, Awards, and Publications

Publications other than abstracts from this project:

(1) Haenszel, W.: Cancer Incidence Among the Foreign-born in the United States. J. Nat. Cancer Inst. 26: 37-132, 1961.

(2) Haenszel, W.: Mortality and Morbidity Statistics on All Forms of Cancer. Presented at U.I.C.C. Symposium on Geographical Pathology, Tokyo, Japan, October 3, 1960.

(To be published in Acta Unio Contra Cantrum.)

Serial No. NCI 54
1. Biometry Branch
2. Demography Section and Clinical Biometry Section
3. Silver Spring, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Studies on Cancer in Connecticut

Principal Investigators: Sidney J. Cutler and William Haenszel

Other Investigators: John Bailar and Fred Ederer

Man Years (calendar year 1960):
Total: 3
Professional: 1½
Other: 1½

Project Description:

(A) Objectives: Provide data on cancer incidence, methods of treatment, and end results. Investigate the effect on incidence of epidemiologic factors such as urban environment, nativity, economic statis, occupation, diet, and personal habits. Study in detail the inter-relationship between the condition of the patient at diagnosis, the therapeutic course, and the end results.

(B) Methods Employed: Hospitals accounting for 94% of the approved general hospital beds in the state participate in a case reporting and follow-up program administered by the State Health Department. Samples of cases reported to the register are also drawn for special study involving the examination of hospital records for additional clinical details, reexamination of histologic specimens, and the collection of histories from patients and relatives.

(C) Major Findings: The study of the long-term survival experience of cancer patients and of changes in survival rates with calendar time has continued with the completion of reports on cancers of the digestive system (stomach, intestine, rectum). Some of the findings are:

(a) Five-year survival rates improved markedly among patients with cancers of the large intestine and rectum. The observed improvement is associated with increased use of surgery and a reduction in operative mortality. The five-year survival rates (adjusted for normal mortality) were - -

	Period of Diagnosis	
	1935-44	1945-54
Large intestine		
Males	19	32
Females	23	39
Rectum		
Males	15	30
Females	23	32

(b) Inspite of increased use of surgery and reduction in operative mortality, five-year survival rates for patients with cancer of the stomach remained low - - approximately 10 percent.

The first two in a series of reports based on an intensive review of hospital records on 5,567 patients with cancer of the uterus have been prepared for publication. Some of the are:

(a) Subsequent experience of 5-year survivors - - Annual survival rates adjusted for normal mortality (annual survival ratio) levelled-off at about 97% for both cervix and corpus. There was little variation with respect to age and stage at diagnosis. Annual survival ratios of patients with cervical cancer were the same whether the original treatment was radiation, surgery, or radiation plus surgery. The group of corpus cancer patients treated with surgery and the group treated with radiation plus surgery had survival ratios only moderately below the expected values. In the group treated with radiation alone, survival rates were significantly below the expected level for at least 20 years; in all groups excess mortality was largely due to late recurrences of uterine cancer. The continuing high mortality for women with corpus tumors treated only by radiation is too large to ascribe to selection of more favorable cases for surgery. It may be due to the effect of radiation itself.

(b) In general the differences in characteristics of patients admitted to small intermediate and large hospitals were not striking. Survival of corpus cancer patients did not seem to depend upon the size of hospital responsible for treatment. There was no association between hospital size and survival rates for cervical cancer patients with localized tumors or for those with remote extension or metastasis. For cervical cancer patients with regional extension, however, survival rates in the large hospitals were considerably higher than those in the small or intermediate hospital.

Intensive follow-up of all patients with breast cancer seen at the Grace-New Haven hospital provided up-to-date information on the survival experience of 79 out of 83 patients originally classified as lost to follow-up. It is noteworthy that their survival experience, subsequent to the original date of last contact, was more favorable than the experience of patients who remained "continuously" under observation. This finding has important implications in statistical methodology.

A pilot study is in progress to evaluate the relationship of the distribution of causes of death to the observed continuing above-normal mortality among breast cancer patients alive 5 years after diagnosis. The study includes evaluation of the accuracy of the cause of death recorded on the death certificate. Very preliminary findings suggest that: 5-year survivors continue to die from breast cancer at a high rate; deaths due to arterio-sclerotic heart disease are much lower than expected; deaths from other cardio-vascular-renal diseases are somewhat higher than expected. These findings are based on death certificate information, the primary cause of death having been coded by a State Bureau of Vital Statistics. We are now comparing these data with information from the hospital records of these patients, and find discrepancies in a sizeable proportion of the cases. No firm conclusions are possible at this time.

(D) Significance to the Program of the Institute: Provides basic epidemiologic information regarding the incidence of cancer and data for evaluating therapy in terms of end results. Also provides means of estimating costs and difficulties of conducting analyses on the larger series of data of the End Results Program.

(E) Proposed Course of Project:

1. The findings of the pilot study now in progress will be used to formulate a series of studies on the relationship between survival time and the distribution of causes of death.

2. The records of patients reported as "untreated" will be reviewed. The relationship of various palliative procedures, such as by-pass surgery, to survival will be studied. The survival experience of patients who refused treatment will be evaluated in order to provide some insight into the natural history of cancer.

Part B included: Yes

Part B: Honors, Awards, and Publications

Publications other than abstract from this project:

Ederer, F., Cutler, S. J., Eisenberg, H., and Keogh, J.R.: Survival of patients with cancer of the stomach, Connecticut, 1935-54. J. Natl. Cancer Inst., 25: 1005-1021, 1960.

Ederer, F., Cutler, S. J., Eisenberg, H., and Keogh, J.R.: Survival of patients with cancer of the large intestine and rectum, Connecticut, 1935-54. J. Natl. Cancer Inst., in press.

Bailar, J. C.: Uterine cancer in Connecticut: Late deaths among 5-year survivors. Submitted to J. Natl. Cancer Inst.

Honors and Awards relating to this project: NONE

Serial No. NCI-58
1. Biometry Branch
2. Demography and Clinical Biometry Sections
3. Silver Spring, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Consultation on cancer registers and application of statistical methods to study of cancer in human populations.

Principal Investigators: William Haenszel and Sidney J. Cutler

Other Investigators: All professional personnel on staffs of both Sections.

Cooperating Units: None

Man Years (calendar year 1960):
Total : 6
Professional: 4
Other : 2

Project Description:

(A) Objectives: To assist other branches of the National Cancer Institute, State and local health departments, and other health agencies by consultation and advice on methodological problems in the maintenance of cancer registers and on the analysis of data collected. This includes the development of statistical tools useful for the analysis of field observations on human populations and their application to specific problems.

(B) Methods Employed: The consultation draws on standard statistical and actuarial techniques and accepted principles for records management, with necessary modifications required for an individual study situation. Many of the problems can be handled by correspondence, but field trips are frequently required. Consideration must be given to the study objectives of the individual investigator or agency, the existing administrative arrangements and the personnel and financial means at hand to implement the program of operations and studies.

Project Description: (cont.)

(B) Methods Employed - (cont.)

Most of the consultation work in this area concerns problems in the integrated use of data from various study techniques: analyses of mortality data, controlled retrospective and prospective studies of selected cohorts, morbidity surveys, screening of populations for precursive signs, and cancer registers. The Branch staff is particularly interested in the methodological problems and issues involved and will continue to do research in this area.

(C) Major Findings: Acting in the capacity of a service organization, the Sections primarily assist other investigators in shaping their opinions and formulating their findings. Sometimes this assistance is acknowledged in publications by the individuals assisted. The principal reports which were prepared with the help of the Branch Staff were:

(a) Collaboration in the analysis of data from the Connecticut Cancer Register is so extensive that it is reported as a separate project.

(b) A report on the characteristics of, therapy received by, and survival experience of 213,000 cancer patients seen in 99 hospitals in the United States. This report was prepared by the End Results Group, Clinical Studies Panel, CCNSC, and was distributed at the Fourth National Cancer Conference, Minneapolis, September 13-15, 1960. In addition to distribution of the aforementioned 119 page statistical report, interpretive reports were presented to the various panels on cancers of the breast, lung, female genital tract, gastro-intestinal tract, genito-urinary tract, skin, and head and neck; and on leukemias and lymphomas.

(c) New methodological approaches to the analysis of survival data and the estimation of the proportion "cured" were developed and incorporated in a doctoral dissertation.

(d) Collaboration with members of the End Results Group in the preparation of the first in a series of projected monographs to be published by NCI. The first monograph, which will be published in 1961, will contain an analysis of trends in the survival of cancer patients; detailed interpretive analyses of the relation of therapy to end results in cancer of the tongue, melanoma of the skin, and leukemia; and a discussion of some methodological considerations in the analysis of patient survival.

Project Description: (cont.)

(D) Significance to the Program of the Institute: Many of the benefits of the consultation program are long-range in nature and consist of setting up record systems and study situations which enlarge the potential study resources of NCI and related organizations. The major activities in the past year are indicated below:

1. Collaboration with the Joint Committee on Staging and End Results Reporting, which is made up of representatives of the major professional medical associations in the United States.

2. Assistance to the Cancer Chemotherapy National Service Center in planning and performing analyses of the data reported to the End Results Section by cooperating cancer registries and in the development of protocols for special studies of selected sites.

3. In cooperation with the Computation and Data Processing Branch, Division of Research Services refinements in the computer program for actuarial analysis of survival data are being worked out to take advantage of the computational flexibility and capacity of the "Honeywell 800".

4. Collaboration with the University of Arizona Bureau of Ethnic Research in establishing population registries for Indian tribes in the Southwest. Such registers are essential to the conduct of many epidemiological studies of cancer among Indians planned as part of the NCI extramural and intramural programs.

5. Work on problems in the integrated use of the various study techniques: analyses of mortality data, retrospective studies of diagnosed cases and matched controls, prospective studies of special cohorts, morbidity surveys, screening of populations for precursive signs, and cancer registers.

6. Liaison with cancer registers in Denmark, England, Finland, France, and Norway has been strengthened and an Ad Hoc Group on International Cooperation in the Evaluation of End Results of Treatment established with representation from 6 countries. The Ad Hoc Group agreed on a set of Rules for reporting end results and made arrangements for presenting international comparisons of survival data at the International Cancer Congress in 1962. Arrangements have been made to process punched cards from the cancer registers in Denmark, Finland and Norway at NIH, in order to insure uniformity of data to be presented at the International Cancer Congress.

Project Description: (cont.)

(D) Significance to the Program of the Institute - (cont.)

7. Data are currently being analyzed in a study of the relationship of adenomatous polyps to carcinoma of the colon and rectum. The study involved the follow-up of two groups of patients: (a) with polyps at initial examination, and (b) without polyps at initial examination.

8. Data collected at the Overholt Thoracic Clinic and at the Ochsner Foundation are being analyzed in order to evaluate the relative merits of lobectomy and pneumonectomy in the treatment of lung cancer.

9. Data collected in a small urban community, Rockford, Illinois, are being analyzed to evaluate the relative merits of limited and radical mastectomy in the treatment of breast cancer. Preliminary findings appear to confirm the report by Smith and Myers, which indicated that no difference in 5-year survival was observed among patients treated by the two different surgical procedures.

(E) Proposed Course of Project: Special attention will continue to be given to international collaboration in studies of survival experience and epidemiology with cancer registers in England and Wales, Denmark, Norway, Finland, France and the Soviet Union.

1. Of particular interest are the detailed data on radiation therapy available in Norway which we hope to use in a study of the long range effects of radiation.

2. A study is being developed on the relationship of radiation dosage and long-term end results in cancer on the uterus. A system for classifying radiation dosage has been developed by H. I. Kohn of the University of California Medical Center.

3. Available data indicate that survival among female patients with lung cancer is considerably better than among male patients, particularly in the sub-group of patients with localized disease treated surgically. Two lines of investigation will be pursued: (a) hospital records will be reviewed to determine whether female and male patients differ with respect to relevant factors such as size and location of the tumor, and (b) the possible association between smoking history and therapeutic end results will be studied.

Project Description: (cont.)

(E) Proposed Course of Project - (cont.)

4. Observed differences in survival among male and female cancer patients will be investigated in attempt to identify prognostic factors. For example, location of tumor in cancer of the tongue, and histological type in cancer of the thyroid.

5. A study is being developed to determine whether endocrine therapy in prostatic cancer significantly increases the risk of developing breast cancer.

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B

Honors, Awards, and Publications

Publications other than abstracts from this project:

Survival Experience of Patients with Malignant Neoplasms. Public Health Service Publication No. 789, 1960, 119 pages.

Cutler, S. J.: A dynamic approach to the measurement of therapeutic end results. To be published in Proceedings Fourth National Cancer Conference.

Cutler, S. J.: A statistical approach to the dynamics of patient survival. Doctoral dissertation, University of Pittsburgh, approved June, 1960.

Reports on end results to be published in Proceedings of Fourth National Cancer Conference:

Hickey, R. C.: Cancer of the breast.

Mersheimer, W. L.: Cancer of the lung.

Latourette, H. B.: Cancer of the female genital tract.

Clayman, C. B.: Cancer of the gastro-intestinal tract.

Moss, N. H.: Cancer of the male genito-urinary tract.

Rohn, R. J.: Leukemias and Lymphomas

Krementz, E. T.: Cancer of the skin.

Linden, G.: Cancer of the head and neck.

Serial No. NCI-51
1. Biometry Branch
2. Experimental Statistics Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Statistical Consulting and Research Service

Principal Investigator: Nathan Mantel and Marvin Schneiderman

Other Investigators: Joan M. Gurian, Lyman B. Crittenden and Max H. Myers

Cooperating Units: None

Man Years (calendar year 1960)
Total: 6½
Professional: 4½
Other: 2

Project Description: Provision of consultative services in statistical design and analysis of experiments, with associated research in statistical methodology.

(A) Objectives: The primary objective is to provide assistance in the design, interpretation, and evaluation of experiments and surveys conducted by researchers at the National Cancer Institute, (and other institutes, on request). This includes services as an associate editor for the Journal of the National Cancer Institute, (Mantel), various referee and review services, and mathematical assistance for development of theoretical models of biological behavior.

(B) Methods Employed: The assistance described above is accomplished using the standard methods of biometry, statistics, and probability, with the necessary modifications required by the problem at hand. Frequently, the standard statistical methods are not sufficient to handle the problem on which assistance is being given, and it becomes necessary for the personnel of the Section to originate new methods.

(C) Major Findings: As a service organization, this Section primarily assists other researchers in making important biological and medical findings, rather than in making such findings directly. Such assistance is frequently acknowledged in the publication of findings of the researchers. Examples of the kind of research problems in which assistance is given are shown below. Occasionally, the nature of the assistance is such as to warrant co-authorship rather than simple acknowledgement. Also new statistical methods originated in this office to handle certain problems are sometimes important enough to warrant separate publication.

(D) Significance to the program of the Institute: The signifigance of the contribution of this Section to cancer research is best illustrated by the problems in which the Section has provided statistical assistance. These include:

(a) models for threshold activity of a carcinogen;
(b) toxicologic properties of specific antitumor agents;
(c) induction of tumors by steroids;
(d) studies of anti-leukemic agents with respect to efficacy and toxicity to host;
(e) investigations of possible synergistic combinations of anti-leukemic agents;
(f) screening programs for anti-tumor agents;
(g) programs for assaying hormonal effects of steroids;
(h) studies on the incidence of lung metastases following surgery or other treatment;
(i) assay of chicken lymphomatosis virus;
(j) study of genetic and other differences in response to chemotherapeutic agents;
(k) metabolic studies of patients;
(l) studies of calcium and strontium incorporation;
(m) fate of tumor cells inoculated into the blood of mice;
(n) effect of radiation of an agent on its activity;
(o) prognostic factors in the survival of cancer patients;
(p) factors involved in hemorrhaging by cancer patients;
(q) safety testing of carcinogens
(r) biochemistry of the antifolics and other potential chemotherapeutic agents;
(s) recovery from chromosomal damage by radiation;
(t) factors affecting spontaneous and induced carcinogenesis;
(u) diagnostic procedures in cancer;
(v) study of mitosis of cells in tissue culture;
(w) genetic, epidemiologic, and environmental studies in human cancer;

(x) studies of cervical cornification;
(y) experimental schistosomiasis and bladder cancer;
(z) significance of metastatic cells in cancer patients following surgery.

(E) Proposed course of the project: The extent to which personnel in this project are involved in providing consulting services relative to the conduct of program activities, as opposed to research by individual investigators, is increasing. For example, in connection with the functioning of the CCNSC such services relate to the Center's programs for assay of hormones, evaluation of drugs and drug combinations against experimental tumors, and clinical evaluation of chemotherapy. Consultation on programs within the field activities area of N.C.I. presently includes the Cytoanalyzer project and the Washington County study. In the contract activities area of NCI, project personnel are involved in the evaluation of potential diagnostic tests for cancer. Advisory service in the design of a program for ascertaining potential carcinogenic hazards has also been provided.

Part B included - Yes

Publications other than abstracts from this project:

Goldin, A.; Venditti, J. M.; Humphreys, S. R.; Shuster, L.; Darrow, R. A. and Mantel, N.: "Advanced Leukemia as a Tool for Chemotherapeutic Studies." Acta Unio Internationalis Contra Cancrum 16: pp 642-650, 1960.

Lieberman, R.; Douglas, J. O. A.; and Mantel, N.: "Production in Mice of Ascitic Fluid Containing Antibodies Induced by Staplylococcus- or Salmonell-adjuvant mixtures". Journal of Immunology, Vol. 84, No. 5, May 1960.

Goldin, A.; Venditti, J. M.; Kline, I.; and Mantel, N.: "Evaluation of Antileukemic Agents Employing Advanced Leukemia L-1210 in Mice. II. Cancer Chemotherapy Screening Data. Cancer Research 20: 382-448, 1960.

Wynder, E. L.; Mantel, N.; and Licklider, S. D.: "Statistical Considerations on Circumcision and Cervical Cancer". Am. J. of Obst. and Gynec. 79, 1026-1030, 1960.

Ketcham, A. S.; Wexler, H.; Kinsey, D. L.; and Mantel, N.: "The Development of Spontaneous Metastases after the Removal of a Primary Tumor. II. Standardization Protocol of Five Animal Tumors. Cancer. (in press).

Mantel, N.; and Patwary, K. M.: "Interval Estimation of Single Parametric Functions." Proceedings of the 32nd Session of the International Statistical Institute, Tokyo, Japan, 1960. (in press).

Mantel, N.: "Principles of Chemotherapeutic Screening". Proceedings of the 4th Berkeley Symposium on Statistics and Probability, 1960 (in press).

Fioramonti, M. C.; Evans, V. J.; Mantel, N.; and Earle, W. R.: "Studies of Nutrient Media for Tissue Cells in vitro. VII. The Effect of the Glucuronic Acid Mixture of NCTC 109 on the Proliferation of NCTC strain 207 and NCTC strain 3145. Am. J. Hygiene, Vol. 72, No. 2, 204-210, September, 1960.

Venditti, J. M.; Humphreys, S. R.; Mantel, N.; Kline, I.; and Goldin, A.: "Evaluation of Antileukemic Agents Employing Advanced Leukemia L-1210 in Mice. III. Congeners of Folic Acid. Cancer Chemotherapy Screening Data VIII. Cancer Research, Vol. 20, No. 10, 698-705, November 1960.

Baer, Paul N.; Crittenden, Lyman B.; Jay, George E., Jr.; Lieberman, Jacob E. "Studies on Peridontal Diseases in the Mouse. II. Genetic and Maternal Effects. Journal of Dental Research (in press).

Lynch, J. M.; Fox, Mary W.; Thayer, W. R.; and Schmit, Germain L.: "Reactions to Invluenza Vaccine Innoculations. A.M.A. Archives of Industrial Health, June 1960, V. 21.

Goldin, A.; Venditti, J. M.; Kline, I.; and Mantel, N.: "Evaluation of Antileukemic Agents Employing Advanced Leukemia L-1210 in Mice. IV. Cancer Chemotherapy Screening Data. Cancer Research (in press).

Crittenden, Lyman B.: "An Interpretation of Familial Aggregation Based on Multiple Genetic and Environmental Factors." Annals of New York Academy of Sciences (in press).

Beye, Henry K., and Gurian, Joan: "The Epidemiology and Dynamics of Transmission of Wachereria Bancroft, and Wachereria Malayi". The Indian Journal of Malariology. (in press).

Arons, Marvin, S.; Smith, Robert R. and Myers, Max H.: "Significance of Cancer Cells in Operative Wounds". Cancer (in press).

Honors and Awards relating to this project:

Nathan Mantel presented invited paper at the meetings of the International Statistical Institute at Tokoyo, Japan.

DIAGNOSTIC RESEARCH BRANCH

January 1, 1960 - December 31, 1960

Although this Branch of the National Cancer Institute did not become an organizational entity until November 14, 1960, most of its activities were carried on throughout the calendar year under the direction of the former Field Investigations and Demonstrations Branch. Among these have been several investigations in exfoliative cytology; the Gastric Cytology Research Project (NCI-86) at the Bowman Gray School of Medicine, Winston-Salem, North Carolina; the Genito-urinary Cytology Project (NCI-89) at the Downstate Medical Center, State University of New York, Brooklyn, N. Y.; the Colon Cytology Research Project (NCI-82) at the Ohio State University Medical Center, Columbus, Ohio; the Uterine Cytology Research Project (NCI-85) at the Woman's Medical College of Pennsylvania at Philadelphia, Pa.; the Uterine Cancer Morbidity Project (NCI-87) at the University of Louisville School of Medicine, Louisville, Kentucky; the evaluation of sputum cytology of Uranium Miners and the field evaluation of the Cytoanalyzer, both of which projects were carried out at the Coffman Research Laboratory at Hagerstown, Maryland.

With the exception of the last two mentioned projects in which the entire investigative group is composed of staff members directly responsible to the Branch, these activities have been carried out as cooperative research investigations (direct field studies) in which staff members work directly with scientists, clinical investigators and physicians in medical schools, universities and cancer centers in various locations in the country.

During the year the Branch (FIDB) established a cooperative research project at the Ohio State Medical School, Columbus, Ohio, in Comparative Human Cytology which is directed toward an investigation of all phases of human cytology as applied to early cancer diagnosis.

All exfoliative cytology studies of cervical cancer, with the exception of the Philadelphia Project, were phased out during 1960.

Other cooperative diagnostic research studies conducted during the year included the investigation of circulating malignant cells in human blood specimens at Bowman Gray School of Medicine (NCI-83); Diagnostic Studies of Gastric Carcinoma (NCI-88) at Duke University Medical Center, Durham, North Carolina; the use of ultraviolet microscopy in the study of malignant disease (NCI-91) at the Pondville Hospital, Walpole, Massachusetts; and an investigation of toxohormone in relation to cancer diagnosis at the Clinical Center, Bethesda, Maryland.

One of the significant methods of operation of the Branch involves the use of contracts to procure diagnostic research. This operational procedure was utilized upon the initiation in the Fall of 1958 of the Diagnostic Development Program as an activity of the Field Investigations and Demonstrations Branch. This activity was continued by that Branch until November 14, 1960, at which time the Field Investigations and Demonstrations

Branch was abolished and the Diagnostic Development Program, along with the cytology investigations, was placed in the newly created Diagnostic Research Branch.

The program is based on the conviction that there must be some interrelationship between the human host and the tumor which is reflected in measurable differences between cancer-bearing and noncancer-bearing individuals. It is believed that these differences are morphological, biochemical, enzymologic, immunologic or hormonal in character. As a consequence efforts have been directed toward determining if such differences exist, can be measured and can be clinically correlated with an early stage of the disease. Essentially all studies are being carried out utilizing human material, body fluids, serum, blood, urine, biopsy and surgical specimens. Data on both normal subjects and cancer patients is accumulated to define the differences between the two populations.

During calendar year 1960, as suggested by Congress, efforts were made to utilize the contract mechanism to a greater extent as a means of increasing research in the diagnostic area. Under the contract procedure new ideas for diagnostic research are generated by the advisors and staff of the Branch. Investigators are approached to determine if they are interested in carrying on the suggested research. If so, the proposals are developed into an adequate protocol and reviewed by appropriate staff members and advisory groups. If found satisfactory, contracts are negotiated and awarded.

Early in 1960 one contract was completed. As of July 1, 1960, 17 contracts totaling approximately $1,000,000.00 were in effect. During the remainder of calendar year 1960 one of the above contracts was concluded, one contract in the amount of $43,222.00 was awarded, and negotiations were being conducted on three additional contracts.

Details of the total activities of the Branch are more specifically outlined in the attached project reports.

Serial No. NCI-81

1. Office of Associate Director for Field Studies
2. Diagnostic Research Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Planning and administration of projects in the field of Diagnostic Research as applied to various human body sites.

Principal Investigator: Dr. R. F. Kaiser

Other Investigators: Dr. S. C. Ingraham, II, Dr. A. W. Hilberg, Dr. M. S. Burstone, Miss Mary M. Bouser, Mrs. Elvira M. May, Mrs. Kathleen C. Fant

Cooperating Units: None

Man Years (calendar year 1960):

Total:	14 1/4
Professional:	5 1/4
Other:	9

Project Description:

The Diagnostic Research Branch, formerly the Field Investigations and Demonstrations Branch, has been engaged in various administrative and scientific functions in relation to studies directed toward earlier diagnosis of human cancer.

Among these activities are:

1) Using sound scientific and biometric principles, to establish and administer cancer diagnostic research contracts.

2) Seeking investigators of recognized scientific standing who are or can be interested in conducting investigations under diagnostic research contracts with the National Cancer Institute.

3) Negotiating and establishing cancer diagnostic research contracts which deal primarily with the application of existing techniques and development of new procedures in the study and recognition of human cancer. (See attached list of contracts in effect.)

4) Utilizing the guidance of special scientific advisors (consultants) for planning and administering contract activities. This would include both PHS and non-PHS professional personnel.

5) Planning policies and objectives of the Branch and developing a program of operation consistent with these policies and objectives.

6) General supervision, statistical evaluation and coordination of cooperative diagnostic research and cytology projects.

7) Participating as requested in cancer research activities of national, state, or local medical and scientific organizations as active members of coordinating committees, advisory committees, etc. Also taking active part in panel discussions and formal presentations as invited by such groups.

Significance to the Program of the Institute: This program should add greatly to our knowledge of the pathogenesis and early diagnosis of human cancer.

Part B included Yes [X] No [ ]

LIST OF ACTIVE DIAGNOSTIC DEVELOPMENT CONTRACTS
DURING CALENDAR YEAR 1960

Contract No. SA-43-ph-	Title
2440	Development of method(s) for determining presence of substances in body fluids and tissues which may be useful in early detection of cancer
2442	Research and development of certain fluorescent dyes (Concluded 12/31/60)
2436	Diagnostic serum profiles in neoplastic and non-neoplastic diseases
2448	An investigation of chromatography and rotatory dispersion of human steroid profiles as applied to cancer diagnosis
2451	Investigation of malignant cells found in peripheral blood from patients with bronchogenic carcinoma
3014	Study of characteristics and growth of human cancer cells isolated from peripheral blood and other body fluids
3015	Fluorescent microscopy of malignant gastric cells
3023	A study to improve methods of diagnosis for endometrial carcinoma
3048	Detection of malignant cells in peripheral blood (Concluded 2/25/60)
3055	Evaluation of the use of peripheral blood cytology techniques for: (1) improved cancer therapy selection, and (2) improved accuracy in prognosis
3096	Study of serum proteins in relation to human cancer
3714	To conduct a research study of the diagnostic clinical correlates of urinary hormones in cancer
3717	Immunologic studies in human neoplastic disease
3716	A study of selected enzyme systems in the peripheral blood of cancer patients

3733 Research investigation on the usefulness of serum and urinary assays in the detection of cancer

3740 Development of chromogenic substrates for histochemical and colorimetric demonstration of enzymes

3756 Research and development of physical methods of dispersing cells for microscopic examination

3751 Investigation of enzyme production by tumorous testis and prostate tissue

3780 A study in immunochemical analysis of body secretions in neoplastic and non-neoplastic diseases

Serial No. NCI-81

PHS-NIH
Individual Project Report
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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Kaiser, R. F., Erickson, C. C., Everett, Jr., B. E., Gilliam, A. G., Graves, L. M., Walton, M., and Sprunt, D. H. Initial effect of community-wide cytologic screening on clinical stage of cervical cancer detected in an entire community. Results of Memphis-Shelby County, Tennessee, Study. Jr. N. C. I., 25: 863-881, 1960.

Courtney, W. B., Hilberg, A. W., Ingraham, 2d, S. C., Kaiser, R. F., Pruitt, J. C., and Bouser, M. M. Field Trial of the Cytoanalyzer: 1,184 specimens analyzed. Jr. N. C. I., 24: 1167-1179, 1960.

Kaiser, R. F., Bouser, M. M., Ingraham, 2d, S.C., and Hilberg, A. W. Uterine Cytology. A preliminary summary of the results of cytological examinations on 600,000 women indicates the efficacy of widespread use of cytology to detect cancer of various organs. Public Health Reports, 75: 423-427, 1960.

Courtney, W. B., Hilberg, A. W., Ingraham, 2d, S. C., Kaiser, R. F., and Bouser, M. M. Modified drop technique: Preparation of smears for automatic, electronic scanning. Jr. N. C. I., 25: 703-711, 1960.

Honors and Awards relating to this project:

None

Serial No. NCI-82
1. Office of Associate Director for Field Studies
2. Diagnostic Research Branch
3. Columbus, Ohio

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Investigation and delineation of the problems of all phases of human cytology as applied to early cancer diagnosis

Principal Investigators: Dr. Emmerich von Haam, Professor and Chairman, Department of Pathology, Ohio State University
Dr. R. F. Kaiser, Chief, Diagnostic Research Branch

Other Investigators: Dr. Gustav J. Selbach, SA Surg., Path., USPHS, MOC
Dr. Donald Kmetz, SA Surg., Path., USPHS, Medical Officer
Dr. Harvey E. Knoernschild, Surg., USPHS, MOC, Colon Survey Project
Dr. Edward M. Miller, Surg., USPHS, MOC, Columbus Cancer Survey Project
Dr. S. C. Ingraham, II, Asst. Chief for Operations, DRB
Dr. A. W. Hilberg, Asst. Chief for Research, DRB
Miss Mary M. Bouser, Nurse Consultant, DRB

Man Years (calendar year 1960):
Total: 39
Professional: 7
Other: 32

Project Description:

Objectives:

(A) A careful and detailed comparison of exfoliated cells from the respiratory tract; gastrointestinal tract; genito-urinary tract; various tissue fluids such as spinal fluids, blood, lymph; breast secretions; and others as indicated, will be carried on by the methods of morphological comparison to biopsy and autopsy tissues.

(B) Similar comparative studies will be made using the methods of cytochemistry, electron microscopy, phase microscopy, etc. with the goal of specific identification of minute details of malignant cells.

(C) Important recent research developments as reported from other laboratories will be checked and repeated as determined to be necessary to establish the usefulness and validity for application to the problems of human cancer cytology.

(D) Development of new methods of processing and interpreting various human cytologic specimens utilizing newer techniques of cytochemistry, microscopy and electronic devices.

(E) Dissemination to other research projects of the Diagnostic Research Branch of research information gained by the above objectives to be utilized for further specific trial in special research programs.

(F) Development of a chemical and/or cytological test for carcinoma of the colon and rectum. This program, if successful, could make practicable earlier detection of these cancers and help decrease the morbidity and mortality from malignancy of the colon and rectum in the general population.

(G) Cytologic material is to be supplied for the clinical evaluation trail of the Cytoanalyzer in Hagerstown, Maryland. Their data will be correlated with results obtained by the standard staining and screening techniques and also compiled to evaluate various epidemiological factors involved in carcinoma of the cervix for evaluation and correlation with the different types of uterine carcinomas found.

Methods Employed: Cytological specimens are obtained by collecting body fluids and scraping epithelial surfaces. These specimens are used to make cytological microscopic preparations, in some instances including Millipore cytology. New Methods in obtaining cytological specimens and their preparations are constantly being revised and evaluated. These procedures are devised in the attempt to develop cytological diagnostic tests for neoplasms of types and locations other than what is currently in the realm of vaginal and colon cytology.

The project is divided into four sections--administration, clinical research, nursing and cytology.

The administrative section compiles and files records and laboratory data and performs other administrative functions necessary to the project.

The clinical section obtains specimens from patients in the University Hospital and its outpatient clinics. In addition, it aids in the correlation of the cytological findings with the state of the disease, treatment employed, symptoms involved and other diagnostic tests.

Certain patients are selected for followup studies, including additional cytological techniques. A complete record of this material is kept on each patient and these records are reviewed and pertinent additional material is added as it becomes available.

The nursing staff is primarily concerned with the mechanics of obtaining specimens. More specifically this entails such duties as assisting with proctoscopic examinations, assisting in the intubation of gastric tubes, the obtaining of clean and catherized urine specimens, obtaining blood by vena puncture, assisting in the aspiration of breast secretions, body fluids, such as asitic fluid and pleural effusions, oral scrapings, sputums, skins, and mass aspirations. In addition, they serve as liaison between the clinical services and the cytological laboratories.

The cytological program is being furnished with the above mentioned specimens from which microscopic preparations are made and examined in an attempt to discover whether cytological examination of these areas is helpful in the detection of neoplastic disease.

This involves constant experimenting in the number of determinations performed upon each lesion, the number of preparations made from each specimen, the type of preparation made and the staining procedures involved.

Patient Material: Patient material is derived from the inpatient and outpatient population of the Ohio State University Health Center and from the patients of the Columbus Cancer Clinic.

Vaginal material was obtained from the above sources and, in addition, from the adult female population of Greater Columbus, Ohio, through cooperation of many private physicians. (The Vaginal Cytology activity was discontinued 6/30/60).

Major Findings:

Comparative Cytology - To date 2700 cases have been evaluated for cytological findings. This entailed the preparation of 7077 slides, including 1700 Millipore filters. The following table illustrates the number of cases studied up to date in each cytology section, along with the number of slides prepared and the results of these studies.

	CASES	SLIDES	POS. PAP CL. 5	SUSP. PAP. CL. 3 & 4	NEG & ATYPICAL PAP. CL. 1 & 2
BREAST	42	86	1	6	35
FLUID	137	693	46	19	72
GASTRIC	172	940	6	20	146
HEMATOLOGY	37	872	2	12	23
ORAL	854	1803	9	17	828
RESPIRATORY	281	1369	44	68	169
SKIN ASPIRATIONS	41	83	1	9	31
URINE	1136	1231	6	28	1102
TOTALS	2700	7077	115	179	2406

To date there is not a sufficient number of cases to either adequately evaluate the accuracy of these methods or to compare these methods with other types of diagnostic tests.

Colon Cytology - In 16793 sigmoidoscopies to date, data is complete in 13390 patients. (There have been 1894 repeat sigmoidoscopies.)

(a) The incidence of colonic polyps has been 7.1% (956 polyps).

(b) Ten percent of biopsied adenomatous polyps have been malignant polyps, or an incidence of 0.3% of all patients examined. (45 cases)

(c) Of all patients examined, 193 (1.44%) have had carcinoma of the colon.

Of the 13390 patients 12356 were examined with sigmoidoscopy cytology (92.3%).

(a) Of the 162 cases of carcinoma of the colon examined by this method, 48% had malignant cytology.

(b) Of the 31 cases of malignant polyps 5 (18%) had malignant cytology.

(c) The incidence of malignant cytology in the benign polyps was 3.1%. An additional 14.5% had atypical cytology.

(d) Of 10012 normal colons only 0.1% had malignant cytology and 2.0% with atypical cells.

Of the 13390 patients in the completed group, 10271 have had their feces examined for blood (77%).

(a) Of the carcinomas of the colon in this group (142) 58% were positive for fecal blood.

(b) Of the known malignant polyps in this group, 23% were positive.

(c) Of the known benign polyps in this group, 12% have had positive blood.

(d) Of the cases in this group with normal colon, 9% have had positive fecal blood.

Results are available from the Millipore filtration of enema returns. A total of 874 cases have been completed.

(a) Of 88 cases of malignancy of the colon, malignant cells have been recovered in 84%.

(b) Of 12 cases of malignant polyps, 20% have had malignant Millipore cytology.

(c) Of the 425 normal colons, there have been 3 false positives.

In comparing the two cytological techniques, the Millipore filter technique appears to be a more reliable method for the detection of carcinoma of the colon above the 25 cm. sigmoidoscopic range. In contrast, the direct smear method is more reliable in lesions lying within range of the sigmoidoscope, but only if seen during sigmoidoscopy.

Vaginal Cytology (Discontinued 6/30/60) - A total of 261,501 slides were received by the time of completion of the project (June 30, 1960) from three major sources:

(a) The Cancer Survey Project Clinic obtained 13,957 slides (5%). This figure also includes specimens obtained in the State Mental Hospital and from several separate industrial and commercial organizations where special arrangements were made to offer the cytology screening test to employees.

(b) The majority of slides (190,944 or 73%) came from private physicians in Franklin County. Included in this group are

slides from five clinics which were established in all the larger Columbus hospitals and which functioned similarly to the Cancer Survey Project Clinic. Over half of all active physicians in Franklin County participated in the project. Most of the others have no gynecological practice.

(c) The remainder of the slides (56,600 or 22%) came from various outpatient clinics of the Ohio State University Health Center, and from the Cancer Clinic.

The total number of cases on which slides were received is 113,758. This represented almost 50% of the adult female population of the community. The difference between this figure and the number of slides received represents cases where both vaginal aspirates and cervical scrapings were received on the same patients, and on cases where repeat, first recall, and second recall examinations were done; 44,009 cases had two or more examinations at annual intervals. Third examinations were received on 16,137 patients and 2,467 had four or more examinations.

A summary of the results of all of the cytologic interpretations is as follows:

Negative	98.4%
Unsatisfactory	0.7%
Suspicious	0.9%

A total of 523 cases of malignancy were detected to date. The overall rate of detection during this period was 4.58 cases of uterine cancer per 1,000 women examined, or one case among every 218 women. The prevalence rate on the initial screening was 3.98 per 1,000. This decreased to 1.50 per thousand on the first rescreening and to 0.24 per thousand on the second rescreening.

The recall program progressed very satisfactorily. Based on the number of cases in our files one year previously, the first recall followup was 49%.

Over 11,000 specimens were supplied to the Hagerstown, Maryland Cytoanalyzer Project.

Record cards were microfilmed and sent to the statistical unit of the Diagnostic Research Branch. Analysis of these cases is now in progress.

Significance to the Program of the Institute: This study by developing methods for detecting and diagnosing cancer in its early stages, will, if successful, offer a means whereby many types of cancer might be more readily controlled in the general population.

Proposed Course of the Project:

Comparative Cytology - Records and data cards are being reviewed for accuracy and completion, and will continue to be throughout the course of this project. It is anticipated that within the next calendar year we will study 6,000 cases by examining approximately 15,000 slides.

During this period we will also make a concerted effort to followup the cases we have studied in order to definitively evaluate the accuracy of our findings.

Colon Cytology - Records and data cards are being reviewed for accuracy and completion in anticipation of the project's closure 6/30/61. It is anticipated that 18,000 sigmoidoscopies will have been performed by that time.

Vaginal Cytology - The study was concluded on June 30, 1960 following a four-year program. Detailed analysis of the results is presently in progress.

Part B included Yes [x] No [ ]

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

The papers "The Diagnostic Value of Fecal Blood Tests in the Detection of Cancer and Adenomas of the Colon" by Drs. Alan B. Cameron, Harvey E. Knoernschild and Robert M. Zollinger, and "Results of Millipore Filtration of Colon Washings in Malignant Lesions of the Large Bowel" by Drs. Harvey E. Knoernschild, Alan B. Cameron and Robert M. Zollinger were presented before the Association of Surgeons of the Colon in Miami Beach, Florida, June, 1960, and will be published in the American Journal of Surgery, January, 1961.

The paper "The Millipore Filtration Technic for Colon Washings" by Drs. Raymond J. Thabet and Harvey E. Knoernschild was published in the Technical Bulletin of the Registry of Medical Technologists, July, 1960, and reprinted in the American Journal of Clinical Pathology, August, 1960.

The paper "The Value of Cytological Studies During Proctosigmoidoscopy Examination for the Detection of Carcinoma of the Colon" by Drs. Alan B. Cameron and Harvey E. Knoernschild was read before the Surgical Forum of the American College of Surgeons in San Francisco, October, 1960.

A scientific exhibit of the "Cancer Survey Projects" was prepared and presented by Drs. Harvey E. Knoernschild, Edward Miller, Raymond J. Thabet and Eileen Macfarlane for the Inter-Society Cytology Council in Chicago, September, 1960.

Honors and Awards relating to this project:

None

Serial No. NCI-83
1. Office of Associate Director for Field Studies
2. Diagnostic Research Branch
3. Winston-Salem, North Carolina

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Peripheral Blood Project - Winston-Salem, N. C.

Principal Investigator: Dr. John C. Pruitt

Other Investigators: Dr. Robert P. Morehead, Dr. Henry F. Mengoli, Dr. A. W. Hilberg, Dr. Samuel C. Ingraham, Dr. R. F. Kaiser

Cooperating Units: Bowman Gray School of Medicine and North Carolina Baptist Hospital

Man Years (calendar year 1960):
Total : 5 3/4
Professional: 2
Other : 3 3/4

Project Description:

Objectives: This is a research project for the purpose of examining human blood specimens for circulating malignant cells and investigating immunologic techniques for destroying those cells, preventing their growth, or utilizing the host-defense mechanisms as a basis for a diagnostic method.

An attempt is being made to answer the following questions:

1) What percentage of patients with cancer have cancer cells circulating in the peripheral blood?

2) What is the relationship of malignant cells in the peripheral blood and prognosis?

3) What percentage of patients have malignant cells in the veins draining their tumor sites at the time of surgery?

4) Does manipulation of a tumor at the time of surgery cause a spraying of malignant cells into the blood stream?

Project Description: (cont.)

5) Do any types of cancer shed malignant cells into the blood early enough and with enough regularity to make the test of diagnostic value?

6) Are cancer cells shed into the blood in a continuous stream or intermittently?

7) Is there any way to determine if the cancer cells identified in the peripheral blood are viable? Is this related to prognosis?

8) What is the effect of the different chemotherapeutic agents on malignant cells in the peripheral blood?

9) What is the effect of infection in patients with cancer on the number and character of malignant cells in the peripheral blood?

10) Does host resistance developed by a person to his own cancer frequently prevent living cancer cells in the peripheral blood from growing at another site? How is this resistance mediated?

11) Can one's natural resistance to the growth of his tumor be potentiated by immunologic methods?

12) Can the hos-defense mechanisms be utilized to provide the basis for a diagnostic test for malignancy?

Methods employed: Blood specimens are processed by the Streptolysin-O technique and stained by the Papanicolaou stain prior to screening for circulating malignant cells.

Immunological evaluation includes serum electrophoresis, soluble tumor homogenate electrophoresis, and column chromatography of soluble tumor homogenates. In addition the following tests are performed.

I. General Immunity Tests.

A. Properdin
B. Complement Level
C. Lysozyme Level
D. Tissue Antibody Level
E. Heterophile Antibody Level
F. Direct Coombs
G. Indirect Coombs

Project Description: (cont.)

II. Specific Immunity Tests.

A. Complement - fixation
B. Precipitin
(a) Macro
(b) Micro
(1) Ouchterlony
(2) Oudin
(3) Immuno-electrophoresis

C. Tannic Acid Erythrocyte Agglutination
D. Shultz-Dale Test
E. Anti Globulin Consumption Test

III. Cytotoxic Tests

A. Trypan blue
B. Oncolysis

Patient Material: The patient material is provided by cooperation of the physicians of the North Carolina Baptist Hospital in Winston-Salem, North Carolina.

Major Findings: Malignant cells may be found in the circulating blood earlier and in greater numbers than was previously suspected. In certain cases the surgeon in unavoidable malipulation of a tumor knocks off a large number of malignant cells into the circulating blood. Most frequently, however, there is not a significant difference in pre-manipulative and post-manipulative specimens.

Significance to Bio-Medical Research and the Program of the Institute: The ultimate results of the studies being conducted at the Peripheral Blood Project should provide useful information about the biology of tumors, problems of hematogenous metastasis, and host-tumor relationships.

Proposed course of the Project: Two to three years.

Part B included Yes [ ] No [X]

Serial No. NCI-86

1. Office of Associate Director for Field Studies
2. Diagnostic Research Branch
3. Winston-Salem, North Carolina

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: A detailed investigation of gastric cancer utilizing exfoliative cytology for case selection.

Principal Investigators: Dr. Robert P. Morehead, Dr. Smith Foushee, Dr. Fritz R. Dixon, Dr. Sidney Girsh, Dr. William Courtney, Dr. Howard H. Bradshaw

Other Investigators: Dr. R. F. Kaiser, Dr. S. C. Ingraham, II, Dr. A. W. Hilberg, Miss M. M. Bouser

Cooperating Units: Bowman Gray School of Medicine, Wake Forest College, Winston-Salem, North Carolina

Man Years (calendar year 1960):

Total	9 1/4
Professional	3
Other	6 1/4

Project Description:

Objectives: The field research investigation using the methods of exfoliative cytology for study of human gastric cancer is engaged first in evaluating known methods of gastric specimen collection, then in studying pathogenesis, course of progression and response to therapy of gastric cancer.

Methods Employed: Methods of approach include a study of patients with even minimal symptoms referable to the stomach or upper gastrointestinal tract, as well as those without symptomatology who are known or found to be achlorhydric, such as patients with known or suspected pernicious anemia. In this manner a study of cellular morphological details in a variety of gastric lesions as well as comparison of these details to normal gastric epithelium and known gastric cancer cells is possible in many cases. The morphology of exfoliated gastric cells is further correlated with the histological material obtained from surgically excised gastric tissue in the same patient. Biochemical studies on gastric secretion are also being investigated in an attempt to facilitate gastric cancer patient identification from the general population. In addition, hematological, physiological, and anatomical methods of possible preselection of human patients are planned.

Project Description: (cont.)

Patient Material: Patient material is derived from the inpatient and outpatient population of the Medical and Surgical Services of the Baptist Hospital in Winston-Salem, North Carolina.

Major Findings: Table I

Gastric Cytology

Total number of gastric washings from July 15, 1958 thru October 25, 1960.

	Number of Washings	Percent
Unsatisfactory (Class 0)	237	23.7
Negative (Class I and II)	700	70.0
Suspicious or Positive (Class III, IV, V)	63	6.3
Total	1,000	

One probably false positive case (Class IV) although still unconfirmed, later reverted to Class II on repeated washings. The complete breakdown of the actual incidence of gastric disease in these cases is not yet available. The meaning of the high percentage of unsatisfactory specimens is difficult to evaluate. If the normal stomach exfoliates few cells as has been postulated by other investigators, then one may account for the high number of unsatisfactory gastric washings.

In 23 cases proven to be gastric cancer (surgical and autopsy data), 17 (74%) were suspicious or positive (Class III, IV, V). The three cases of gastric cancer in this group which had negative cytology (false negative) were two cases of malignant lymphoma of the stomach, and one case of poorly differentiated carcinoma of the stomach.

In six cases with the clinical diagnosis of gastric cancer (inoperable cases), 5 (83%) had had suspicious or positive (Class III, IV, V) gastric washings.

Exfoliative cytology is proving to be a valuable tool in the diagnosis of gastric cancer. It appears to have merit in confirming the presence of malignancy in those cases which are equivocal by other methods of diagnosis.

Project Description: (cont.)

Significance of the Program to the Institute: This study by developing methods for detecting and diagnosing gastric cancer in its early stages, will, if successful, offer a means whereby this type of cancer can be more readily controlled in the general population.

Proposed Course of the Project: This project is designed to run a 5-year course.

Part B included Yes [ ] No [X]

Serial No. NCI-84

1. Office of Associate Director for Field Studies
2. Diagnostic Research Branch
3. Hagerstown, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Exfoliative cytology including 1) field evaluation of the performance of the Cytoanalyzer. 2) Evaluation of morphology of cells found in sputum of uranium miners.

Principal Investigators: Dr. Charles C. Spencer, Dr. Albert W. Hilberg, Dr. William B. Courtney

Other Investigators: Dr. R. F. Kaiser, Dr. S. C. Ingraham, II, Mr. Paul Montague, Miss Mary M. Bouser, Miss Sarah J. Smith

Cooperating Units: Columbus, Ohio, Cancer Survey Project
Walter Reed Army Hospital
Johns Hopkins Hospital
Hagerstown, Washington County Health Department
Airborne Instruments Laboratory
Colorado Plateau Industrial Physicians and Health Departments
Atomic Energy Commission
Division of Occupational Health
Radiological Health Division

Man Years (calendar year 1960):

Total	:	9 1/2
Professional	:	2
Other	:	7 1/2

Project Description:

Objectives: 1) To evaluate the performance of the Cytoanalyzer in presorting human vaginal smear preparations for technician screening.

2) To test and develop new special techniques for preparing and staining vaginal smears for scanning by the Cytoanalyzer.

Project Description: (cont.)

3) To evaluate morphology of cells from the bronchial tree found in sputum specimens from uranium miners; also to develop a satisfactory technique for collecting sputum and transporting and processing specimens for examination for pulmonary cytology examination.

Methods Employed: Cytoanalyzer readings are compared to screening results by cytotechnicians on smears prepared from the same vaginal aspirate. Vaginal aspirates are prepared for the Cytoanalyzer using a modified drop technique and Feulgen Stain. Special studies are being made of machine reproducibility and accuracy.

Sputum specimens are collected and processed in the field and shipped to the laboratory for staining and morphologic evaluation by technicians, cytologists and pathologists. For shipping, the smears are covered with Carbowax and wrapped in wax paper, then packed in mailing tubes.

Patient Material: Selected examinees of the Columbus, Ohio, Cancer Survey Project. Patients of the Gynecology Service of Walter Reed Army Hospital. Patients of Johns Hopkins Hospital. Uranium miners on the Colorado Plateau.

Major Findings: The results from the second clinical trial of the Cytoanalyzer, involving over 11,000 vaginal smears indicates that the Cytoanalyzer can prescreen (make unnecessary any examination by human screeners) approximately 37% of all negative smears. At this prescreening level it incorrectly classifies as negative not more than 10% of the suspicious and positive smears. A practical, standardized, modified drop technique has been developed to prepare smears for use in the Cytoanalyzer.

In the sputum cytology phase of this activity, approximately 500 smears (of about 8,000 collected) were screened, evaluated and studied. Followup studies were recommended on the suspicious and positive sputums found. A contract was awarded to expedite examination and evaluation of the remaining, unexamined sputums in this study.

Significance to Biomedical Research and the Program of the Institute: Application of an automatic electronic scanner in sorting cytologic smears, if successful, can facilitate prescreening of large volumes of such material with possible earlier cancer case

Project Description: (cont.)

identification and treatment. Because of its enormous speed and accuracy in scanning cells and intracellular structures, this machine should also be very valuable in basic cytological research.

Study of the uranium miner sputum specimens is expected to furnish important information about the effect of exposure to uranium mine atmosphere on the pulmonary bronchial mucosa.

Proposed Course of the Project: A re-evaluation of the instrument's capabilities will be made to define its limits of resolution and discrimination, accuracy and reproducibility. This will permit a more exact description of the type of material and type of preparation suitable for use in the Cytoanalyzer. It will evaluate the instrument's potential for basic cytological research.

The participation of this laboratory in the uranium miner sputum study is being terminated because of transfer of the entire uranium miner study to the Bureau of State Services.

Part B Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Courtney, W. B., Hilberg, A. W., Ingraham, S. C., Kaiser, R. F., Pruitt, J. C., and Bouser, M. M.: Field Trial of the Cytoanalyzer; 1,184 Specimens Analyzed. Jr. NCI 24: 1167-1179, 1960.

Courtney, W. B., Hilberg, A. W., Ingraham, S. C., Kaiser, R. F., and Bouser, M. M.: Modified Drop Technique: Preparation of Smears for Automatic, Electronic Scanning. Jr. NCI 25: 703-711, 1960.

Honors and Awards relating to this project:

Serial No. NCI-85
1. Office of Associate Director for Field Studies
2. Diagnostic Research Branch
3. Philadelphia, Pennsylvania

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: A study in an industrial population of cervical cancer using vaginal cytology for preliminary case selection - Philadelphia, Pennsylvania

Principal Investigators: Dr. I. N. Dubin, Dr. M. R. Dunn, Dr. Bernard Behrend, Dr. M. D. Pettit, and Dr. Edwin B. Hodge

Other Investigators: Dr. S. C. Ingraham, II, Dr. A. W. Hilberg, Miss M. M. Bouser, Dr. R. F. Kaiser, and Miss Anne Baranovsky

Cooperating Units: Woman's Medical College of Pennsylvania, Departments of Pathology, Preventive Medicine & Gynecology

Man Years (calendar year 1960):
Total : 22
Professional: 3
Other : 19

Project Description:

Objectives: Approximately forty thousand employed women in the Philadelphia area were examined by means of vaginal cytology smears. Two, repeat annual examinations are being made on all examinees who can be located on successive years. This study will provide data on 1) the detection of cervical cancer in the early stages and 2) various factors in the etiology and pathogenesis of the disease, such as:

a) prevalence and incidence of cervical cancer
b) inter-relationship of such lesions as atypical metaplasia, intraepithelial carcinoma and invasive carcinoma of the cervix
c) whether intraepithelial carcinoma always progresses to invasive carcinoma and over what period of time.
d) whether intraepithelial lesion seen during pregnancy is the same lesion as in the non-pregnant woman, and
e) role of age, ethnic background, socio-economic status, gynecologic and obstetrical history, and geographic locale.

Objectives: (cont.)

The reasons for studying employed women are:

a) this is a stable group, with relatively little turnover of population
b) the economic status is more determinable
c) this group is better organized, and it is easier to get to and work with this group
d) one can utilize already existing occupational medical health services

Methods Employed: This project is the product of a cooperative arrangement between the National Cancer Institute, Diagnostic Research Branch, and the Woman's Medical College of Pennsylvania. The National Cancer Institute provides the immediate professional, technical and administrative staff, as well as the necessary supplies and equipment. The Woman's Medical College provides the laboratory space, with its permanent fixtures and maintenance, and the professional supervising direction of the study.

There is an Advisory Committee representing the participating departments of the Woman's Medical College (viz, the Departments of Pathology, Gynecology, and Preventive Medicine), the Philadelphia County Medical Society, the Philadelphia Branch of the American Cancer Society, the College of Physicians, the Philadelphia Pathological Society, the National Cancer Institute, a clinical cytologist, and additional representatives of local teaching institutions.

There is also a Special Committee on Preventive Medicine, comprising physicians from the Woman's Medical College, Philadelphia physicians active in occupational medicine, and a representative of the Industrial Nurses Association.

Contact with employed women is made by the physicians on the staff of the Project, through the Occupational Physician of a given industry. There is no publicity to the lay public at large. The employees are given the choice of:

1) going to their private doctor for a complete physical examination and vaginal cytology smear (to be sent to the Cytology Laboratory), or

2) having a smear taken by trained personnel of the project, at the industrial site. In all cases a card with pertinent data is sent to the project with the smears. All reports on the cytologic specimens go to the woman's private doctor, who in turn sends a report to the patient and acquaints her with its significance. Patients with

Methods Employed: (cont.)

suspicious smears are referred to their private physicians who base definitive diagnosis and treatment on accepted clinical and biopsy studies. Followup data is sought through the cooperation of a) personnel of the project, b) occupational physicians and nurses, c) private doctors, and d) pathologists in other institutions who make available slides from biopsy specimens for study by the pathologists of the project.

The data obtained is coded and recorded in a medical statistical form (1819-9) as utilized by other cytology units. These cards are microfilmed for statistical analysis by the biostatisticians in Bethesda.

Patient Material: Employed women in selected industries in the City of Philadelphia were utilized during the first year of operation. During 1959, activities were expanded to include the surrounding counties of Bucks, Delaware and Montgomery.

Major Findings: Since screening began in November 1957, 34,338 women have been examined by vaginal smears; 13,713 have been screened for a second time; and 3,468 to date have had three yearly screenings. Of these numbers, 238 have had suspicious or positive vaginal smears. Of these 238 women, 168 have had cervical biopsies which have been sent to us for examination. In this group there have been 63 intraepithelial carcinoma of cervix; 8 i.e. with suspicion of microinvasion; 12 i.e. with microinvasion; 14 invasive carcinoma of cervix. Nonmalignant lesions included: 14 severe dysplasia (possible i.e. CA); 17 moderate atypism of cervical epithelium and 25 mild atypism or chronic cervicitis. There were 10 inadequate biopsy specimens.

Significance to the Program of the Institute: The results of this study will be added to the information being obtained in other cytologic studies. Consolidated data are expected to furnish insight into pathogenesis of uterine cervical cancer, course of development of the lesion and its incidence and prevalence in the adult female population.

Proposed Course of the Project: Preparations for closing the project are in progress with seven employees leaving February 1, 1961. All screening will stop December 31, 1961 and a followup period of six months with a skeleton staff to finish is anticipated. Final closeout date will be approximately June 30, 1962.

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

None

Honors and Awards relating to this project:

Marvin W. Stafford, Supervisory Technician, was given appointment as Assistant in Cytology within the Pathology Department of Woman's Medical College.

Serial No. NCI-87

1. Office of Associate Director for Field Studies
2. Diagnostic Research Branch
3. Louisville, Kentucky

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: A uterine cervical cancer morbidity study in Louisville, Kentucky

Principal Investigators: Dr. William M. Christopherson, Mrs. Winifred M. Mendez

Other Investigators: Miss M. M. Bouser, Miss Anne Baranovsky, Dr. James E. Parker, Dr. R. F. Kaiser, Dr. A. W. Hilberg

Cooperating Units: University of Louisville School of Medicine

Man Years (calendar year 1960):

Total	4½
Professional	1
Other	3½

Project Description:

Objectives: 1) To determine the impact of cervical cytology on the incidence of cervical carcinoma in a large urban area when the cytologic examination is offered to women through their family physician, the health facilities in industry and the clinics in the city hospital.

2) To determine the incidence of symptomatic cancer of the cervix among the non-cytologically tested population of women in the Louisville area.

3) To provide a baseline for comparison and evaluation of cytology results obtained from the Louisville Cancer Survey Project.

4) To provide adequate assessment of the meaning of casefinding or incidence rates for cancer of the cervix on successive screenings.

5) To help discover symptomatic cases between screenings among women in a cervical cytology study, some of which may have been missed during the previous screening.

Objectives: (cont.)

6) To help clarify the natural history of the disease.

Methods Employed: 1) Identification and clinical description of all cases of uterine cancer diagnosed, treated, or deceased in Jefferson County for the years 1953-54-55, to establish a baseline of uterine cervical cancer incidence in the population prior to the Cancer Survey Project.

2) Identification and clinical description of all cases of uterine cervical cancer diagnosed, treated, or deceased in Jefferson County during 1956-57-58, the years during which the Cancer Survey Project was functioning in order to study the effect of a cytology project on the incidence of cervical cancer.

3) Identification and clinical description of all cases of cervical cancer diagnosed, treated, or deceased for the years 1959-60-61, the years following the termination of the cytology project in order to study the result of the cytology project on the incidence of cervical cancer and the effect of cervical cytology on the course of the disease and of survival rates in the cytology population.

4) Identification of the uterine cancer cases will be accomplished by visiting all the local hospitals where uterine cancer is diagnosed or treated, all private pathologists and radiologists. Case records will be summarized and matched with records of patients in the cytology project population.

All death certificates registered in Jefferson County listing uterine cancer as the primary or contributory cause of death will be listed and will be matched with cases identified from all other sources and with patients in the cytology population.

5) Overall incidence rates for cervical cancer and rates for the cytology and non-cytology populations will be studied for the periods prior to the cytology project, during the cytology project and post-project period.

6) Survival rates for uterine cancer cases in the cytology and non-cytology populations will be compared.

7) A card was designed for making abstract of case history records. A marginal punch keysort card was designed, and a code for classifying data has been written.

Patient Material: All uterine cancer cases diagnosed, treated, or deceased in Jefferson County, Kentucky.

Major Findings: The study was initiated June 1959. Collection of data for the years 1953-1958 is virtually completed; the resident cases for this period have been collated on marginal punch cards and are ready for coding. No collating has been done on nonresident cases. Collection of data on the 1959 uterine cancer cases has been completed in five hospitals.

Significance to the Program of the Institute: The morbidity study should assist in evaluating cervical cytology as a tool for early diagnosis of cervical cancer. On the basis of patient records the value of cervical cytology in the initiation of early treatment and consequent anticipated improved survival may be appraised.

In conjunction with material from other similar studies the data from this project will help to clarify the natural history of the disease.

Proposed Course of the Project: Continued collection of data on uterine cancer cases in the study area for the projected years. Recording collated information and coding on marginal punch keysort cards. Continued search for missing pieces of information on already identified cases. Analysis of data as sufficient material is organized, and population figures are available.

Adding case history data for the years 1959-60-61 as the patient records become available.

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Mendez, Winifred M.: The Nurse Epidemiologist in Cancer Research. Presented before the 1960 Nursing Workshop of the School of Nursing, Catholic University of America, Washington, D. C., and to be published in the Proceedings of The Workshop, Spring 1961.

Honors and Awards relating to this project:

None

Serial No. NCI-88

1. Office of Associate Director for Field Studies
2. Diagnostic Research Branch
3. Durham, North Carolina

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Diagnostic Studies of Gastric Carcinoma

Principal Investigator: Dr. Jane G. Elchlepp

Other Investigators: Dr. J. C. Hijmans

Cooperating Units: Duke University Medical Center
Durham VA Hospital
North Carolina Memorial Hospital
Lincoln Hospital

Man Years (calendar year 1960):
Total: 3
Professional: 1
Other: 2

Project Description:

Objectives:

1) To assay possible changes in gastric secretions and tissues by chemical and immunochemical procedures to determine their significance to the diagnosis and prognosis of gastric carcinoma.

2) To assay possible changes in other gastrointestinal secretions and in blood by chemical and immunochemical procedures to determine their significance to the diagnosis and prognosis of gastric carcinoma.

3) To detect and evaluate possible sequential functional processes in the development of, and reaction to, malignant changes of gastric tissue.

Methods Employed:

In general, the procedures enumerated below are to be used in the study of gastric secretions, blood, salivary secretions, intestinal secretions, and cancerous and noncancerous gastric tissues.

1) Electrophoretic studies of proteins and glycoproteins and immunoelectrophoresis studies of blood group mucoids,

2) Chemical studies of proteins glycoproteins, hexosamine, fucose, sialic acid and blood group mucoids,

3) Blood group studies of titre levels and secretor status, and

4) Histologic studies with particular emphasis on tagged antibody methods.

Patient Material:

Material is obtained from patients at Duke University Medical Center, Lincoln Hospital, Durham VA Hospital, and North Carolina Memorial Hospital.

Major Findings:

Electrophoretic studies of salivary secretions have not proved successful for use on a mass screening basis. It is probable that some concentrated work on technical procedures might alter this, but for the present this must be considered a qualitative technique that is too cumbersome to use in studying large patient numbers.

Electrophoretic studies of tear proteins have shown significant alterations in patterns for diseased patients as compared to normal individuals. We sample approximately 90-100 patients and obtained satisfactory results on 53 patients (164 studies). Most of the unusable results were obtained while we were working out collection and laboratory procedures and we are now able to obtain about 90-95% usable results on current collections. The significant alterations occurred mostly with certain clinical stages in inflammatory and bleeding diseases of the gastrointestinal tract but a gastric carcinoma was among this group.

Data has not yet been summarized on other phases of the project work.

Significance to the Program of the Institute:

Information obtained from the project outlined may be of value in developing a profile of diagnostic studies which would be of value in the detection of gastric cancer. Stress is placed upon the blood group substances in this study because of repeated correlations noted in statistical studies between occurrence of blood group A and gastric carcinoma.

Proposed Course of the Project:

1) The positive results noted above in the tear protein electrophoretic studies have suggested the necessity for intensifying and extending studies on this secretion. Publication of the preliminary survey results is in preparation.

2) Time must be taken to review and evaluate material collected during the past year.

3) Additional personnel (at medical level) should be considered to take advantage of material which can be obtained as result of an expanding departmental program of studies on gastrointestinal cytology and gastric physiology. (See outline submitted October 1960 for details.)

Part B included Yes [X] No [ ]

Serial No. NCI-88

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

None

Honors and Awards relating to this project:

Dr. Elchlepp was promoted as of July 1, 1960, to Assistant Professor of Pathology, Duke University Medical Center.

Serial No. NCI-89

1. Office of Associate Director for Field Studies
2. Diagnostic Research Branch
3. Brooklyn, New York

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The use of exfoliative cytology in the detection of genito-urinary cancer.

Principal Investigator: Dr. Frank C. Hamm

Other Investigators: Dr. Donald P. Peyser
Mr. Marta Garret
Dr. Frank Watson
Dr. Phillip Cabaud

Cooperating Units: Downstate Medical Center
State University of New York
450 Clarkson Avenue
Brooklyn, N. Y.

Man Years (calendar year 1960):

Total:	2 1/4
Professional:	3/4
Other:	1 1/2

Project Description:

Objectives:

1. To determine the accuracy of exfoliative cytology as an adjunctive diagnostic tool in carcinoma of the prostate gland; with specific emphasis placed on its usefulness in the detection of early prostatic carcinoma in the patient with no apparent clinical or laboratory signs of the disease.

2. To evaluate the significance of cytologic examination of the urine following massage with two points in mind:

a) A chance to compare the technical aspects of a direct prostatic smear with smears made from urine following prostatic massage.

b) The opportunity to evaluate cytologically the bladder and upper genito-urinary tract.

3. To study methods for enhancing the flow of prostatic secretion; including the effect of various drugs on this function.

Methods Employed:

Study Group. The patients obtained for study will be males age 50 and above who enter a general medical clinic. They may or may not have any urologic symptomatology; however, the presenting complaints and salient symptoms are noted on a clinical record.

Along with this more random group is a parallel series of patients (matched by age with the study population) who are referred to the private office of a urologist at the Brooklyn Hospital with definite urologic symptomatology or for a routine periodic examination. Both groups are being sampled in a similar fashion and similar record cards are being kept; however, the source of each patient is carefully noted on each card.

Collection of Specimen: Each patient has a complete rectal examination and prostatic massage. Two smears are made from the prostatic secretion and a urine following massage is collected. Four slides are made from the urinary sediment.

Data: All patients are classified in a prescribed way determined by the clinical and cytological evaluation. Any patient having a clinical and/or cytological suggestion of malignancy is urged to enter the hospital for further evaluation and treatment, where necessary. The nonsuspicious patients clinically and cytologically are seen at least twice per year.

Major Findings:

Kings County Hospital:

Total number of new patients seen from June 15, 1960, through December 21, 1960	-	308
Approximate number of slides examined	-	1900
Number of new patients clinically suspicious and histologically proved to have carcinoma (Only one false negative in these 7. The rest were suspicious or positive on cytologic examination.)	-	7

Number of patients clinically suspicious found to have a negative needle biopsy - 8
(Only one patient had suspicious cytology. The rest had negative cytology.)

Number of patients clinically nonsuspicious with normal cytology - 283

Number of patients detected having bladder carcinoma from our study - 2

Number of patients clinically nonsuspicious for carcinoma of the prostate having atypical cytology and ultimately having the histologic diagnosis of carcinoma of the prostate - 1

This last patient was given a deferred diagnosis as his cytologic interpretation which signifies marked atypia of the cells; however, the cytologist is prevented from going further since it has been very difficult so far to establish whether these are atypical suspicious cells of prostatic origin or cells arising from other areas of the genito-urinary tract and having no pathologic significance. More histologic data will ultimately help us solve this problem.

There is also a small group of patients who are clinically suspicious for carcinoma but for one reason or another have not been biopsied.

Recall Follow-up: 59%
(88 patients of 149 recalled returned.)

Unsatisfactory specimens: 24%
(75 unsatisfactory prostatic samples ultimately in 308 patients.)

Brooklyn Hospital:

Total number of new patients seen from June 15, 1960, through December 21, 1960 - 78

Approximate number of slides examined - 500

Number of patients clinically suspicious and histologically proved to have carcinoma - 5
(One false negative. The rest had positive cytology.)

Number of patients clinically suspicious found to have a negative needle biopsy - 5
(One patient had suspicious cytology, class III, in these 5, whereas the rest had normal cytology.)

Number of patients clinically nonsuspicious with normal cytology - 58

Number of patients clinically nonsuspicious with suspicious cytology proved to have carcinoma - 0
(There is one 57 year old man who during a routine examination was found to have a slightly suspicious prostatic area. His prostatic smear was class IV. A needle biopsy was negative and had it not been for the impetus of the class IV smear the Surgeon might have stopped there and followed the patient clinically. A large wedge resection of the prostate with serial sectioning finally revealed carcinoma of the prostate gland.)

Unsatisfactory specimens: 14%
(11 unsatisfactory in 78 patients.)

Combined Totals of Both Hospitals:

Number of patients seen from June 15, 1960, through December 21, 1960 - 386

Approximate number of slides examined - 2400

Ancillary Findings:

1. Our data demonstrated that doing prostatic smears provides no more information than doing cytologic examinations of urines following prostatic massage alone. The urines often demonstrate positive cytology in the absence of positive prostatic smears but almost never is the reverse true in our experience.

2. We find in urinary cytology that the millipore filter technique has an advantage over centrifugation of urines in two situations:

a) The urine with little or no visible sediment.

b) The urine sample which is of a small aliquot.

In the usual urine specimens we find we save more time and have as good cytologic results by just centrifuging the urines and sampling the sediment.

3. An attempt was made to evaluate the secretory response of dog prostates to stimulation by a variety of drugs. Pilocarpine was the only drug to increase prostatic secretion.

In six humans in which 5 mgm. Pilocarpine was given orally, two showed marked increase in secretory response with many more prostatic cells on the post-medication slides compared to the pre-medication slides. Three patients had no change while one had a diminution in prostatic response. There were no untoward effects of this drug given orally at the above dosage.

Significance to the Program of the Institute:

The results of this study should provide data as to the pathogenesis, detection, and treatment of genito-urinary cancer. We hope also to demonstrate the importance of periodic rectal examinations in the male over age 50 with an ultimate desire of reducing the high mortality in prostatic cancer.

Proposed Course of the Project:

A 5-year study is planned.

Part B included Yes / / No /X/

Serial No. NCI-90
1. Office of Associate Director for Field Studies
2. Diagnostic Research Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Clinical Trials Unit

Principal Investigator: Andrew C. Peacock, Ph.D.

Other Investigators: None

Cooperating Units: Clinical Pathology Department, CC

Man Years (calendar year 1960):
Total: 2
Professional: 1
Other: 1

Project Description:

Objectives: To develop tests capable of aiding in the detection of cancers of all sites in early stages of development. The over-all aim is to develop one or more cancer diagnostic tests that will be simple, inexpensive, easily duplicated, and therefore, applicable to large population areas on a mass basis.

Methods Employed: Basic research is employed upon chemical and immunological principles underlying tests described in the past, and principles which aid in the development of new tests. During the past year, the principles upon which some general tests for diagnosis have been based have been evaluated. Modification of these principles has been attempted, and new procedures have been adopted which may aid in the formulation of new tests. Procedures developed are evaluated upon the serum from known cancer patients, and from normal people.

Patient Material: Patients and staff of the Clinical Center.

Major Findings:

Acceleration of blood coagulation: Further work on Stuart-Prower Factor and on prothrombin has indicated that preparations of either of these substances are usually contaminated by small amounts of the other,

and efforts to provide reliable methods for the elimination of these traces has continued. By continuous flow electrophoresis some samples of prothrombin have been prepared with very low Stuart factor content, probably because of destruction of Stuart factor during the process. Other means of selective destruction are being sought.

Toxohormone: The primary aim of this first phase of research on toxohormone has been to devise methods for isolation and assay which would permit more definite statements to be made concerning the distribution of toxohormone in various tissues, both normal and malignant. Methods for isolation of toxohormone described by Nakahara and by Greenfield and Price have been successful when applied to transplantable tumors, but do not appear suitable when used with other tissues. Difficulties encountered are very low solubility of the product, failure of precipitate to appear in early stages of the purification, and variable activity of the products. In addition, we frequently find products which when injected according to the usual schedule, are lethal to the mice, thus preventing assay.

A second general difficulty is encountered with the quantitation of such products as appear to be active. We have been unable, as a rule, to devise any kind of dose-response relationship between the amount of material injected and the subsequent depression of liver catalase.

We have made numerous trials in an attempt to get soluble products by a reliable procedure. In the method currently employed acetone powder of tissue is extracted with hot trichloroacetic acid to remove (by hydrolysis) the nucleic acids and to precipitate the proteins. Toxohormone activity, if present, is found in the precipitate. This material has on occasion been further purified by continuous flow electrophoresis and by chromatography on Sephadex (pore-size gel). By this means, material devoid of nucleic acid and aromatic amino acids have been obtained. These fractions were not toxic and could depress liver catalase, but a suitable reference (i.e. mg. protein or mg solids) could not be reliably estimated. Even with this material there was no evident dose-response relation. We have been about equally successful in preparing toxohormone from normal and tumor tissue by this means, but often fail to find activity from tumor tissue.

We have lately been investigating the ability of these extracts to inhibit catalase in vitro where it is possible to relate the extent of inhibition to the amount of material used. We find, contrary to the Japanese literature, that many of our toxohormone preparations do show such inhibition. These results are too fragmentary at present to be discussed further.

Serum alkaline phosphatases: Work on the problem of distinguishing the several serum alkaline phosphatases has been resumed. Alkaline phosphatases may be separated by a chemical fractionation involving alcohol, by chromatography on DEAE cellulose, and by electrophoresis. We have sought to find ways in which the enzymatic activity of these fractions differ. We have investigated Michaeles constants, optimum Mg concentration and effect of excess substrate. Surprisingly, we find these constants to be very similar (Km = 10^{-3}M, Mg 10^{-3}M, excess substrate inhibits at about 0.1 M). It appears from the chemical fractionation that the alkaline phosphatases in bone disease and in liver disease are very different. In chromatography, however, the differences do not appear to be related to the disease state so directly. We are currently investigating the significance of altered serum proteins in liver disease as a factor in this separation.

Significance to the Program of the Institute: The development of any test that would aid in the detection of early cancer in one or more sites would be of great value to the clinician. With strong suggestive evidence at hand that the patient has a cancer, the present diagnostic procedures could be intensified in studying this patient; and there would be impetus for the development of further diagnostic procedures. It is evident that the development of such tests would be of great practical value in combating the cancer problem.

Proposed Course of the Project: We propose to continue the study of in vitro inhibition of catalase as well as to continue our attempts to describe the distribution of toxohormone in normal and malignant tissues. We will undertake a systematic study of the electrophoretic distribution of alkaline phosphatases in serum of normal and sick people, with a view to clarifying the effect of disease on serum levels of these enzymes.

Part B included Yes / X / No / /

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Williams, G. Z. and Peacock, A. C.: Ultra Violet Studies of Tetrazolium Reduction in Living Cells. Exptl. Cell Research, Supplement 7, 131 (1959).

Peacock, A. C., Williams, G. Z., and Mengoli, H. F.: Rapid Electronic Measurement of Cell Volume and Distribution. J. Nat. Cancer Inst. 25: 63 (1960).

Peacock, A. C.: Formation of Thromboplastic Activity with Russell's Viper Venom. J. Lab. Clin. Med. (in press).

Honors and Awards relating to this project:

None

Serial No. NCI-91

1. Office of Associate Director for Field Studies
2. Diagnostic Research Branch
3. Walpole, Massachusetts

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The use of ultraviolet microscopy in the study of diseases of the blood including conditions with blood borne metastases

Principal Investigator: Dr. John B. Vander

Other Investigators: Dr. R. F. Kaiser

Cooperating Units: Pondville Hospital, Walpole, Massachusetts
Framingham Heart Project, National Heart Institute

Man Years (calendar year 1960):
Total : 2½
Professional: 1
Other : 1½

Project Description:

This project is directed primarily in the investigation of the use of ultraviolet microscopy as a diagnostic tool in the study of the leukemias and related diseases.

It may be roughly divided into three parts, all of which involve microscopic examination of specimens prepared in different ways and stained with various fluorescent dyes (e.g., A & B isomers of Fluorescein Isothiocyanate and A & B isomers of Rhodamine Isothiocyanate).

1) Tumor cells -- Bone marrow preparations and peripheral blood specimens have been studied intensively in an effort to quickly identify tumor cells in these specimens. Although tumor cells can occasionally be found, the yield is much less than that using other techniques. It is believed this is due to the fact that the cytoplasm of circulating tumor cells in relatively inactive, contains little RNA, and therefore gives only a feeble secondary fluorescence. This is in

Project Description: (cont.)

accord with the hypothesis that most of the cells are not viable and hence do not produce widespread metastasic lesions as might be expected.

2) Leukemias -- This is a study of white blood cells which we can now demonstrate very beautifully and with very clear visualization of the details of the cytoplasm and nucleus in leukemic cells. A high content of RNA is easily demonstratable in the cytoplasm. It is believed that a continued study along these lines may lead to a method which will aid in the diagnosis of leukemias.

3) Anemias -- Although the anemias which are associated with the leukemias are being studied, other types are also being studied. The greatest emphasis is being placed on this phase of the study at the present time since severe intractable anemia is one of the most frequent causes of death in leukemic patients. Using the ultraviolet technique, we are now able to detect reticulocytes in peripheral blood with a great deal more accuracy than is possible by conventional methods. In leukemic patients, we are able to demonstrate changes in immature red blood cells which, to our knowledge, have not been previously described as occurring in peripheral blood. These changes consist of changes in the ribonucleic acid (RNA) and desoxynucleic acid (DNA). These changes will be studied in patients receiving anti-leukemic treatment such as Myeloran, and X-ray therapy.

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Freymann, J. G., Vander, J. B., Marler, E. A., and Meyer, D. G.: Prolonged Corticosteroid Therapy of Chronic Lymphocytic Leukemia and the Closely Allied Malignant Lymphomas. Brit. J. Hematology, Vol. VI, No. 3, 303-323, July, 1960.

Vander, J. B., and Johnson, H. A.: Chronic Lymphatic Leukemia and Multiple Myeloma in the Same Patient. Ann. Int. Med., Vol. 53, No. 5, 1052-1059, November, 1960.

Honors and Awards relating to this project:

Elected to Fellowship in the American College of Physicians, December, 1960.

PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NCI-140
1. Assoc. Director for Field Studies
2. Epidemiology Branch
3. Los Angeles, California

Part A.

Project Title: A study of cancer of the uterine cervix in the Memphis-Shelby County population

Principal Investigators: Mary Walton, M. D., J. William Lloyd, Benno K. Milmore, M. D., and Alexander G. Gilliam, M. D.

Other Investigators: Cyrus C. Erickson, M. D. and Douglas Sprunt, M. D.

Cooperating Units: The Department of Pathology of the University of Tennessee College of Medicine and the Memphis-Shelby County Health Department

Man Years (calendar year 1960):

Total: 4
Professional: 1
Other: 3

Project Description:

Objectives:

1) To evaluate mass use of the vaginal aspiration cytological test as a screening method for the detection of cancer of the uterine cervix in a general population.

2) To study epidemiologically the relationship between carcinoma-in-situ and invasive cancer of the cervix.

Methods Employed:

Both of the general objectives depended upon the initial cytological examination of a large proportion of the adult female residents of Shelby County, with repeated rescreening at approximately annual intervals thereafter. A morbidity survey covering the 2-1/2 years prior to the study provided a baseline for evaluation of the effect of cytological screening on the occurrence of uterine cancer in the

Part B. included Yes [X] No [ ]

community. Beginning in July of 1952, cytological examinations were offered without charge to the women of the county. As of the closing of the laboratory, nearly 2/3 of the female residents over 19 years of age (almost 80% of those between 20 and 49 years old) had a satisfactory initial examination, and over 40% of these had one or more rescreening examinations. Concurrently all new cases of uterine cancer among residents, both screened and not-screened, were recorded by morbidity survey, which was continued thru 1958. The unsuspected prevalence of in-situ and invasive carcinoma of the cervix detected at first screening and identified at subsequent rescreenings provides prevalence and incidence rates of in-situ and invasive disease among the screened population. Incidence among non-participants and among women who had had one or more examinations before a diagnosis not related to a screening was measured by morbidity survey. These data permit eipdemiological study of the relationship between in-situ and invasive carcinoma as well as comparison of relative frequency of occurrence of uterine cancer among the different racial and socio-economic segments of the population, as well as evaluation of the sensitivity and specificity of mass vaginal cytological screening tests.

Patient Material:

The adult female population of Memphis-Shelby County area.

The three major sources for vaginal aspiration specimen collection were: (1) private physicians, (2) regular daily clinics held at a local hospital, and (3) annual temporary clinics held in large industries, hotels, banks, schools, etc.

Major Findings:

First screenings done thru June (152,000), and rescreenings thru October of 1957 (90,000) were coded and punched. These included non-residents and unsatisfactory observations. Effective numbers are accordingly smaller. There were 114,846 satisfactory first screenings and 50,764 rescreenings among resident women over 19 years of age.

Unforseen difficulties have delayed seriously the final analysis of results. At present, most of the essential tabulations of the population experience following initial screening are still not available for study.

Certain important findings are evident, however. The marked improvement in proportion of cases of cervical cancer in clinical Stage I at diagnosis has been reported (Part B).

Imcomplete tabulations confirm the earlier suggestion that a substantial proportion of insitu lesions must regress and cannot progress to invasive disease. Age differences in the relationship of in-situ to invasive carcinoma are suggested as well as other factors of potential clinical significance. These cannot be studied in detail until the tabulations

presently requested are available.

Significance to Cancer Research:

Important inferences regarding the natural history of in-situ and invasive lesions of the cervix will be available from this study.

Proposed Course of Project:

Data collected in 1960 were:

(1) Missing information on morbidity survey thru 1958;

(2) Follow-up information on cases identified as carcinoma in-situ;

(3) Continued follow-up of cases of invasive carcinoma, with record of available information on clinical status and mortality thru June of 1960.

The Memphis Unit was closed on June 30, 1960. Analytical work continues in Bethesda and in Los Angeles as tabulations are made available. A second paper, "The Relationship of Carcinoma in-situ to Invasive Carcinoma of the Uterine Cervix - Evidence from the Memphis Study" has been completed except for final minor revisions anticipated after conference with the principal collaborating pathologist, Dr. Erickson.

Work continues on other aspects of data to be presented in the planned series of reports. No statistical clerical assistance was available for this project between July 1 and early December. At present, nothing final can be done until the tabulations of population experience are received. Then, the progress will be in proportion to the effective man-hours made available for organization of the data. It seems desirable that the major work on the project be completed at least within calendar year 1961. To do this, the tabulations now requested must be available by March 1, after which more adequate statistical clerical help will be required for prompt completion.

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Kaiser, Raymond F.; Erickson, Cyrus C.; Everett, Bennett E., Jr.; Gilliam, Alexander G.; Graves, Lloyd M.; Walton, Mary, and Sprunt, Douglas H.: Initial effect of community-wide cytologic screening on clinical stage of cervical cancer detected in an entire community. Results of Memphis-Shelby County, Tennessee, study. J.N.C.I. 25:863-881 (Oct.) 1960.

PHS
Individual Project Report
Calendar Year 1960

Serial No. NCI-143
1. Assoc. Director for Field Studies
2. Epidemiology Branch
3. Los Angeles, California

Part A.

Project Title: Excess Lung Cancer Among Mexican Females

Principal Investigator: Mary Walton, M. D. and John E. Dunn, Jr., M. D.

Other Investigators:

Cooperating Units: Bureau of Chronic Diseases, State of California Department of Public Health, Pathology Department and Cancer Research Unit, University of Southern California School of Medicine

Man Years (calendar year 1960):

Total: 0
Professional: 0
Other: 0

Project Description:

Objectives:

A study of lung cancer mortality among Mexican females in California showed that Mexican-born females have a three-fold lung cancer risk as compared to other California women. No increased risk was found for Mexican males or California-born women of Mexican extraction. The objective of this study is to attempt to determine what factor or factors may be responsible for the increased risk.

Methods Employed:

To obtain information regarding geographic location and characteristics of place of origin, age at migration, and other available history by interview of surviving relatives of cases.

Similar information has been collected about the Mexican female population of California.

Major Findings:

None since 1959 report.

Part B. included yes ☐ No. ☒

Significance to Cancer Research:

The elucidation of factors associated with increased risk of cancer may contribute to the understanding of the pathogenesis of the disease.

Proposed Course of Project:

An effort to obtain information from immigration folders about 107 Mexican-born females who died of lung cancer prior to the fall of 1956 proved unproductive.

It is proposed that another attempt be made to obtain certain data about these cases plus those identified subsequently to date by attempting to locate and interview surviving relatives of these patients. Data to be sought would include age at migration, State of birth and residence prior to migration, with specific questions as to population density (urban-rural), and geographic and climatic characteristics (mountain-desert). While incidence rates by State for migrants could not be calculated, it is possible that the distribution of places of former residence might suggest or virtually eliminate this variable as a possible significance. While occupational history probably would be inadequate, something is known of occupational patterns in certain locations. If, for example, most of the individuals migrated as adults from areas where manufacture of pottery or glass has been the major industry for generations, and where young girls are employed in these processes (Puebla, Guadalajara), it might be possible to investigate further. Differences would exist in exposure to smoke in cooking between rural and urban areas and between colder areas at higher altitudes and hot desert or coastal locations. A skillful interviewer should have little difficulty in obtaining this kind of information from people of Mexican culture.

Work on this project will be resumed as soon as Mrs. Winifred M. Mendez is released from her present assignment in Louisville, Ky., with the Diagnostic Research Branch, NCI, and transferred to Los Angeles for duty with the Epidemiology Branch. It is hoped that this transfer will be effective in July, 1961.

PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NCI-145
1. Assoc. Director for Field Studies
2. Epidemiology Branch
3. Los Angeles, California

Part A.

Project Title: Achlorhydria Survey of the Japanese Population of Los Angeles by the Tubeless Gastric Analysis Method.

Principal Investigator: Charles Carpenter, M. D. and John E. Dunn, Jr. M. D.

Other Investigators: Members of the Medical Staff of the Japanese Hospital

Cooperating Units: California State Department of Public Health

Man Years (calendar year 1960):

Total: 1
Professional: 1/2
Other: 1/2

Project Description:

Objectives:

The immediate goal is to determine the frequency of achlorhydria among the Japanese people of Los Angeles, California, as compared to Mexican and Caucasian control groups. Stomach-cancer mortality among Japanese of Continental United States and Hawaii is more than 3 times as great as among the white population. Other studies among Caucasians suggest a relationship between achlorhydria and risk of developing gastric cancer.

Methods Employed:

Japanese people were selected for study because their gastric cancer mortality experience is unusually high. The acid-producing ability of the stomach is determined by the tubeless method, utilizing azure-A ion-exchange resin. Blood type is also determined.

Physicians are notified of their patients' test results. When the result is "indeterminate" or "no free HCl present," they are asked to urge the patient to have the test repeated.

Patient Material:

Private Japanese patients of the Japanese physicians on the staff of the Japanese Hospital in Los Angeles, patients of the Los Angeles County General Hospital, and some voluntary non-patient participants (both Japanese and non-Japanese) are examined.

Part B. included Yes ☐ 142 No ☒

Major Findings:

Grinspoon and Dunn (J.N.C.I. 22:617-631, 1959) reported that the frequency of achlorhydria among the Japanese was substantially the same as among Caucasians. Among persons of Mexican origin there was an apparently excessive frequency of achlorhydria.

The latter group has been enlarged to 354 and the excess of achlorhydria persists, the difference between Mexican and Caucasian subjects being statistically significant ($p < .01$). A report on this phase of the study is being prepared.

Significance to Cancer Research:

Development of simple techniques for early detection of gastric cancer would be an important contribution to control of this disease. While tubeless gastric analysis will not directly detect gastric cancer, it may serve as a screening method to identify persons among whom the risk of this disease is high. More elaborate diagnostic procedures can be carried out among small high-risk groups than would be feasible for the entire population.

Proposed Course of Project:

The immediate goal, as described under "objectives," has been achieved and no further testing for achlorhydria is contemplated. Instead, we shall try to find a reason for the unexpected finding of excessively frequent achlorhydria among Mexicans. Dietary habits will be studied to ascertain whether they are correlated with the appearance of achlorhydria. Consultation related to the proposed new course of the project is being obtained from Miss Helen Walsh, Chief, Nutrition Service, California State Department of Public Health.

Because of her skill as an interviewer and her ability to speak Spanish, the hoped-for assignment of Mrs. Winifred M. Mendez to Los Angeles will greatly facilitate the future conduct of this project.

PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NCI-146
1. Assoc. Director for Field Studies
2. Epidemiology Branch
3. Los Angeles, California

Part A.

Project Title: Circumcision and Cervical Cancer

Principal Investigator: John E. Dunn, Jr., M. D.

Other Investigators: George Drake, M. D., Charles Carpenter, M. D. and Mr. Philip Buell

Cooperating Unit: University of Sourthern California School of Medicine
Dr. Ian Macdonald

Man Years (calendar year 1960):

Total: 1
Professional: 2/3
Other: 1/3

Project Description:

Background:

A detailed analysis has been made of the data on circumcision of male partners of cervical cancer patients and controls from the study conducted by Dr. Edward Jones and Dr. Ian Macdonald during the period 1951-55. This analysis showed no difference in the frequency of circumcision of the male partners of cervical cancer patients and controls.

These findings were at variance with those published by Wynder, et al. (Am. J. Obst. & Gynec. 67:1016-, 1954), in which the uncircumcised male was found to be the principal cause of excess of cervical cancer in gentile women as compared to Jewish women. A study carried on by Lilienfeld (J. Nat. Cancer Inst. 27:713-, (Oct.) 1958) came to our attention, in which a surprising discrepancy was found between the statement of men as to circumcision status and the findings on medical examination. A similar study was carried out by Dr. Grinspoon, and then by Drs. Drake and Carpenter in Los Angeles with somewhat similar findings. Further than this it was found that this discrepancy did not exist for Jewish men, circumcision for them being almost always anatomically complete. On the basis of the misclassification men were responsible for in regard to themselves, and assuming women would do no better in evaluating their male partners, it was shown that a case-control study of women might easily fail to show a protective effect of circumcision even though it exists.

Part B. included Yes ☐ No ☒

Our conclusion, then, is that the protection of women from cervical cancer afforded by completely circumcised males is an attractive hypothesis that still lacks proof, and the usual case-control study in which male circumcision is determined from querying women on their male partners is unlikely to discover such a relationship. The definitive study will involve women with cervical cancer who have had only one husband and determining circumcision status by examination.

Objectives:

Determine whether circumcision status of males is related to the occurrence of cancer of the cervix among their sexual partners.

Methods Employed:

Interviews by physicians of cervical cancer patients who had been married once only and examination of their husbands for circumcision status. Controls will be selected to match on age, race, social status, and whether ever pregnant.

Patient Material:

Private patients of Dr. Ian Macdonald in Los Angeles.

Patients at Gynob Clinic in San Diego.

Major Findings:

A group of private patients kindly made available by Dr. Ian Macdonald was studied. Sixty-eight cases and controls were interviewed and 31 husbands were interviewed and examined in order to ascertain the degree of circumcision. Ten husbands were circumcised, 9 uncircumcised, 9 were partly circumcised and 3 were not examined. At the San Diego Gynob Clinic, 56 cervical cancer patients and 24 husbands have been interviewed. Seventy-one control patients having negative cervical cytology and 38 of their husbands were interviewed. The clinics sizeable accumulation of cases of early invasive cervical cancer and carcinoma in situ, as identified by cytology, provide a good source of cases which we plan to continue to utilize in 1961.

Significance to Cancer Research:

This study will confirm an important lead to etiology of cervical cancer as well as a simple control measure if circumcision of the male is found to be protective against the disease.

Proposed Course of Project:

At present arrangements have been made to continue interviewing cervical cancer patients and suitable controls from the practice of Dr. Purvis

Martin in San Diego. When these are completed patients from other physicians in the Gynob Group will be interviewed.

Cervical cancer mortality is only half as frequent among Japanese women in California as among other women in the state. Japanese men were examined to determine frequency of circumcision. Preliminary evidence indicates that this is infrequent among Japanese men, only 13 of 54 Japanese males being completely circumcised. This study will be continued.

PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NCI-141
1. Assoc. Director for Field Studies
2. Epidemiology Branch
3. Berkeley, California

Part A.

Project Title: Relationship of Cancer Occurrence and Socio-economic Factors

Principal Investigator: John E. Dunn, Jr., M. D. & Philip Buell

Other Investigator: Lester Breslow, M. D.

Cooperating Unit: San Francisco Health Department

Man Years (calendar year 1960):

Total: 1/3
Professional: 1/3
Other: 0

Project Description:

Objectives:

Evaluation of socio-economic factors in relation to the occurrence of cancer generally and specific sites.

Methods Employed:

1) Death certificates coded by census tracts were available from the San Francisco Health Department for the years 1950-1953.

2) Two sociological studies of San Francisco were available in which census tracts had been classified on three dimensions - Family Status, Social Rank, and Segregation.

3. Sex-age-site specific death rates for San Francisco as a whole were calculated and applied to the appropriate census tract populations to get expected cancer deaths by site and sex. These were compared with observed deaths from tracts grouped according to the socio-economic variables being studied.

Major Findings:

Principal interest centered on the Social Rank and Family Status variables. Significant correlations were found as follows:

Part B. included Yes [X] No [ ]

1) For males negative correlations were found with Social Rank and cancer of all sites, upper G. I. tract, stomach, and pancreas. There were no positive correlations.

2) For females Social Rank was negatively correlated with cancer of the G. I. tract, stomach, and cervix uteri. The usual positive association for breast cancer was not found.

3) For males Family Status showed some positive association for cancer of the prostate.

4) For females Family Status was positively correlated with cancer of the stomach and pancreas and negatively correlated with cancer of the cervix uteri.

5) The interaction of Social Rank and Family Status is evident for cancer of the stomach where the highest rates for both sexes was in the Low Social Rank-High Family Status areas. This is also true for cancer of the pancreas in females. Cancer of the cervix uteri is concentrated in the Low Social Rank-Low Family Status area.

Significance to Cancer Research:

1) A number of studies of socio-economic factors in relation to cancer have been made--first in England, then Denmark, and more recently in this country. Socio-economic gradients have been found fairly consistent for some sites; less consistent for others. For example, cancer of the breast in females does not show a gradient in our study. There have been other departures from European studies in this country and what dimensions best demonstrate them.

2) In this study three sociological dimensions were considered for measuring gradients. For some sites the interaction of two dimensions appears to indicate they reinforce each other.

Proposed Course of Project:

This project has been completed and results have been published. See Part B.

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Buell, P.; Dunn, J. E., Jr., & Breslow, L.: The occupational-social class risks of cancer mortality in men. J. Chronic Diseases 12: 600-621 (Oct.) 1960.

PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NCI-144
1. Assoc. Director for Field Studies
2. Epidemiology Branch
3. Berkeley, California

Part A.

Project Title: Occupation and Lung Cancer

Principal Investigator: John E. Dunn, Jr., M. D.

Other Investigators: Various staff members, Bureau of Chronic Diseases
California State Department of Public Health

Cooperating Units: California State Department of Public Health

Man Years (calendar year 1960):

Total: 1/3
Professional: 1/3
Other: 0

Project Description:

Objectives:

In a prior case-control study of lung cancer patients, in which detailed occupational histories were gotten, it was found that certain occupations appeared with greater frequency in the lung cancer patients than in the controls. Among the suspect occupations were: welders, various occupations using asbestos in their work, painters, cooks, marine engineers and firemen, and electric bridge crane operators. These findings are being investigated further in the present study.

Methods Employed:

The present study is a prospective study in which populations of workers engaged in these occupations are being assembled to be followed for future lung cancer experience. Since populations of 5-10,000 men 35-64 years of age are desired, it was necessary to use a mailed questionnaire. Besides identifying information, the questionnaire asks for pertinent information regarding the kind of work engaged in, the nature of the exposures, length of time in the occupation, and smoking history. Union organizations offered the best opportunity for reaching the numbers desired. They have proved to be most cooperative, and mailing response has ranged between 85-90 per cent with three mailings.

Part B. included Yes [X] No [ ]

Both Northern and Southern California are included in the study. At present, data collection is limited to the southern part of the state and has been completed.

In addition to the suspect occupations, a control population of utility workers has been assembled.

Lung cancer, other cancer (and possibly other causes of mortality) will be determined in these groups by checking all cancer deaths for the State against these assembled populations.

Queries are being sent to the appropriate physicians for diagnostic confirmation of all lung cancer deaths.

Significance to Cancer Research:

Occupations found to carry increased lung (or other cancer) risk will need to be studied for identification of the specific cause. From this could come specific control measures.

Major Findings:

Death clearance was completed through 1958 and a preliminary analysis made of the data. There have been 139 lung cancer deaths that have occurred during the period covered in the analysis. Several occupations appear to be associated with an excess of lung cancer. However, the magnitude of the excess is not great - being in the range of 1.5 to 2 times that experienced by the male population generally. One of the major problems in this and any other study searching for variables associated with lung cancer occurrence is the masking effect of such a powerful and nearly universal factor as cigarette smoking. It is possible that excesses of the magnitude found might represent factors that would increase the lung cancer risk five-fold if it could be examined in the absence of smoking.

Proposed Course of Project:

The final analysis will not be undertaken until five years of follow-up death clearance has been completed for each of the occupational groups being studied.

The principal investigator was retired from PHS on Nov. 1, 1960, and is now the Cancer Consultant for the California State Dep't. of Public Health. Future work on this study will be conducted by the CSDPH.

Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Dunn, J. E., Jr., Linden, George, Breslow, Lester: Lung Cancer mortality experience of men in certain occupations in California. Am. J. Pub. Hlth. 50:1475-1487 (Oct.) 1960.

PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NCI-142
1. Assoc. Director for Field Studies
2. Epidemiology Branch
3. Atlanta, Georgia

Part A.

Project Title: Oral cancer in relation to vitamin deficiency, use of snuff, and other factors

Principal Investigator: William R. Vogler, M. D.

Other Investigators: J. W. Lloyd and B. K. Milmore, M. D.

Cooperating Unit: Emory University School of Medicine

Man Years (calendar year 1960):

Total: 5/6
Professional: 1/3
Other: 1/2

Project Description:

Objectives:

1) To determine whether a relationship exists between the use of tobacco, avitaminosis, ill-fitting dentures, and other factors, and the development of epidermoid carcinoma in the oral cavity.

Methods Employed:

1) A questionnaire has been completed on all new patients seen in the Robert Winship Clinic between January 1956 and July 1957. In addition, all old patients with mouth cancer and/or leukoplakia were included. About 2000 questionnaires have been completed.

2) All of the records of mouth cancer patients have been reviewed in the hope of finding some common denominator.

3) Appropriate statistical tests have been applied to determine the significance of differences between disease groups.

Patient Material:

Patients at Robert Winship Clinic in Atlanta.

Part B. included Yes [ ] No [X]

Major Findings:

1) Cancer of the mouth is seen more frequently in women here than in other parts of the country.

2) It appears that the consumption of chewing tobacco and snuff is much higher among the cancer patients than among the controls.

Significance to Cancer Research:

This investigation, as far as it proceeded, hints at a specified relationship between use of snuff and-or avitaminosis and the occurrence of cancer of the upper gastrointestinal tract.

Proposed Course of Project:

Except for publication of results, this project has been completed. The manuscript indicated below is being typed and will be submitted for publication.

Vogler, William R.; Lloyd, J. William, & Milmore, Benno K.: A retrospective study of etiological factors in cancer of the mouth, pharynx, and larynx.

PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NCI-147
1. Assoc. Director for Field Studies
2. Epidemiology Branch
3. Bethesda, Maryland

Part A.

Project Title: Research in epidemiologic method, stimulation of epidemiologic research in non-Service agencies, and development of sources of field data related to specific epidemiologic questions.

Principal Investigators: A. G. Gilliam, M. D., B. K. Milmore, M. D. and J. William Lloyd, M.Sc.

Other Investigator: John E. Dunn, Jr., M. D.

Cooperating Units:

Man Years (calendar year 1960):

Total: 2
Professional: 1
Other: 1

Project Description:

Objectives:

To examine recorded epidemiological evidence in cancer for its validity and to develop more adequate epidemiological tools for cancer investigation.

To stimulate the inauguration of epidemiological studies of cancer among people and agencies having pertinent material, the exploitation of which will contribute to an understanding of human cancer; to provide technical assistance in the design of studies and analysis, of such material; and to explore sources of material directly available to us and suitable for answering specific questions which appear particularly important.

Methods Employed:

Adaptation and modification of standard epidemiological methods already of demonstrated usefulness in communicable diseases research, and extension and application of these methods to cancer study, including the application of such methods to published data for test of validity of conclusions drawn.

Part B. included Yes [X] No [ ]

Through contacts made at meetings of national professional associations, lectures to medical and public health students and exploitation of personal friendships with persons in many agencies, to stimulate and assist in organizing studies of basic epidemiologic material not directly available to the Service. By the same means obtain access to data for our own study.

Major Findings:

Further consultation was given for a study of cancer in a large free-living population among which there are restrictions against the use of alcohol, tobacco, and coffee. (Gilliam & Milmore)

Cancer epidemiology lectures or seminars were conducted at medical or public health schools at Ann Arbor, Mich., Pittsburgh, Johns Hopkins, Univ. of California, and at the Communicable Disease Center. (Gilliam & Milmore)

Consultation given several investigators in the design of studies relating to possible hazards of diagnostic radiation. (Milmore & Gilliam)

Participated in a site visit for the American Cancer Society in connection with a request for research funds. (Milmore)

Participated in conduct of a national cooperative study of leukemia. (Gilliam & Milmore)

Consultation was given to several non-government investigators in design of epidemiologic studies.

Extension of the "Dunn Model" for epidemiological study of relation, if any, between "ca-in-situ" and invasive cancer of the cervix. (Gilliam, Milmore, Dunn, & Lloyd)

Provision of statistical consultation and work to other Sections of the Branch. (Lloyd)

Significance to Cancer Research:

Any theories of causes of cancer in humans, from no matter what scientific discipline they come, will remain inadequate until they meet the test of consistency with epidemiological facts. Hence, development of more precise and powerful epidemiological tools is of considerable importance to cancer research.

The future, and broad usefulness, of research in this field depends partly on exploitation of material not directly available to us and on recruitment and training of future workers.

Proposed Course of Project:

To be exploited as new opportunities arise.

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project.

Dunn, J. E. : Relation of carcinoma in situ to invasive carcinoma of the cervix uteri. Proc. of 4th Symp. on Math. Stat. and Probability. Berkeley, Calif., July 22, 1960.

Dunn, J. E., Jr.: The epidemiological aspects of cervical carcinoma as revealed by cytologic study. J. Internat. College of Surgeons 34:710-725 (Dec.) 1960.

Gilliam, A. G.: Book review of "Morbidity from cancer in the United States" by Harold F. Dorn and Sidney J. Cutler, Public Health Monograph No. 56, Washington, D. C., U. S. Gov't. Print. Off., 1959. Am. J. Pub. Hlth. 50:901-902 (June) 1960.

PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NCI-148
1. Assoc. Director for Field Studies
2. Epidemiology Branch
3. Bethesda, Md.

Part A.

Project Title: Cancer in a closed population

Principal Investigators: A. G. Gilliam, M. D., B. K. Milmore, M. D. and J. William Lloyd, M.Sc.

Other Investigators:

Cooperating Units: A large metropolitan Police Department

Man Years (calendar year 1960):

Total: 3/4
Professional: 1/4
Other: 1/2

Project Description:

Objectives:

Primary objective is to measure risk of death from all causes, but more particularly from cancer of the lung, in a closed employee population with possible exposure to gasoline and diesel engine exhaust fumes in excessive amounts.

Methods Employed:

Analysis of employment records of about 60,000 men employed between 1906 and 1953; determining causes of death in those dead, and comparing death rates in this population with that for other men in the same city.

Major Findings:

Age adjusted relative frequency comparisons suggest that lung cancer might be excessive in this group. However, exact measures of risk await computation of person-years at risk.

Part B. included Yes ☐ No ☒

Significance to Cancer Research:

Determination of lung cancer mortality in an industrial population exposed to a suspected carcinogenic agent.

Proposed Course of Project:

Records are currently being checked for possible errors and duplications. This phase should be completed by March 1, 1961 if sufficient clerical help is available. Cohort life tables for determining observed and expected mortality should be finished by September, 1961.

PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NCI-149
1. Assoc. Director for Field Studies
2. Epidemiology Branch
3. Bethesda, Maryland

Part A.

Project Title: Geographic, Racial, and Other Factors in Distribution of Cancer

Principal Investigators: A. G. Gilliam, M. D., Benno K. Milmore, M. D. and J. William Lloyd, M. Sc.

Other Investigators: William J. Pendergrast, M. D., Atlanta, Georgia and John E. Dunn, Jr., M. D., Berkeley, California

Cooperating Units:

Man Years (calendar year 1960):

Total: 3
Professional: 1
Other: 2

Project Description:

Objectives:

To determine geographic, racial, and other factors influencing risk to cancer.

Methods Employed:

Comparison of recorded deaths attributed to cancer of specified sites among selected population groups. Geographic, racial, and other factors are being studied. Trends in cancer mortality are also under investigation. When available, cancer incidence data are also utilized.

Major Findings:

Four manuscripts were completed. See Part B.

Significance to Cancer Research:

The epidemiological method depends on the demonstration of differences in risk in people with different characteristics, environment, etc. Thus, the results of this project are descriptive and provide information for use in tests of consistency with a variety of theories of cancer causation, as well as leads for further exploration.

Part B. included Yes [X] No [ ]

Proposed Course of Project:

To be continued and expanded in similar direction with other cancers as personnel and facilities become available.

Data on the trends and geographic distribution of several cancer have been completed. Reports of these analyses will be prepared.

PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NCI-149

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Gilliam, A. G.: Geographic distribution and trends of leukemia in the United States. Unio Int'l. Contra Cancrum Acta 16:1623-1628, 1960.

Gilliam, A. G.; Milmore, B. K. and Lloyd, J. W.: Trends of mortality attributed to carcinoma of the lung: The declining rate of increase. (Accepted June 17, 1960 for publication in Cancer.)

Gilliam, A. G., & Walter, W. A.: Trends of mortality from leukemia in the United States, 1921-1955. The Year Book of Cancer. 1959-1960. The Year Book Publishers, Inc., Chicago, 1960 (pp. 401-404).

Pendergrast, W. J.; Milmore, B. K., & Marcus, S. C.: Thyroid cancer and thyrotoxicosis in the United States: Their relation to endemic goiter. (Accepted June 9, 1960, for publication in J. Chronic Diseases).

Serial No. NCI-153
1. Office of Associate Director for Field Studies
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Geographic Distribution of Cancer in the U. S.

Principal Investigators: P. A. Lawrence and B. E. Carroll

Other Investigators: J. W. Lloyd and S. Shusterman

Cooperating Units: None

Man Years (calendar year 1960):
Total: Less than 1/3
Professional: Less than 1/3
Other: Less than 1/3

Project Description:

Objectives: To map the distribution of mortality due to malignant disease within the United States and to see if any geographic patterns exist which might be correlated with variations in known geological, topographical, or other features of the country.

Methods Employed:

1) This project was initiated several years ago by calculation and mapping of relative frequency of cancer as a cause of death in each of the counties of the United States for a 3 year period--1949, 1950 and 1951. Raw data sources were the National Office of Vital Statistics and Bureau of the Census. Comparisons among counties within each state revealed interesting and suggestive geographical patterns which frequently extended across several state boundaries.

2) Availability of the 650 computer permitted production of more accurate measures of cancer mortality over a longer time period for all counties in the United States. These measures are in the form of mortality ratios standardized for age, race and sex, computed for a 5 year period centered around 1950. These data have now been plotted onto maps and the geographical patterns which have emerged are under intensive study by comparisons with published maps showing broad geophysical features of the country.

3) Work is now going forward on a further refinement of the mortality ratios to take into account such factors as urbanization, physician density, and proportion of foreign born population. The 650 computer will be used for these refinements.

4) An earlier phase of this study was based on special tabulations of data on mortality from oral and respiratory cancer in 15 states for a 10-year period. Standardized mortality ratios were computed for counties within these states, adjusted for age, race, and sex. This phase of the study was described completely in project NCI-116 for 1959.

Major Findings: From spot maps prepared so far, it is apparent that human cancer occurrence and environment, in a broad sense, have a measurable positive correlation. The most recent plot of all the standardized mortality ratios reveals some provocative geographical patterns which seem to correlate surprisingly well with certain broad geophysical features of the United States. For example, in the eastern half of the United States (east of the 102nd Meridian) there appear two broad and different patterns of cancer experience:

1) Elevated cancer experience seems to occur in the northeast portion of the country composed of all the New England States, New Jersey, and all of the states which border on the Great Lakes. (A similar pattern has been indicated in several older studies based on less detailed data and for different time periods.) This area contains approximately 48% of the United States population, the greatest concentration of industrial activity, and the most highly urbanized regions of the country. Both urbanization and some forms of industrialization have long been known to be associated with elevated cancer experience; however, within this broad region there are many counties and larger areas of low urbanization and industrialization which show the elevated cancer experience characteristic of this broad region. In addition to the obvious regional characteristics, there is at least a suggestion of a possible long range meteorological factor provided by the fact that this region of high cancer experience is bounded surprisingly well by a line marking the maximum southern extension of the great ice sheets which penetrated the North American continent during the last period of glaciation.

2) Lower cancer mortality experience is revealed in a broad region composed of all the states lying south of the Ohio River and extending westward from the Atlantic Seaboard to a rather sharply defined western boundary which runs diagonally from the southern tip of Texas to a point in central Missouri. Within this broad pattern (extending sometimes across state boundaries) in which elevated cancer experience is indicated. Notable examples are to be found (a) along the Appalachian Highlands, (b) in the phosphate mining region of

central Florida, and (c) in a roughly triangular patch enclosed by a line extending northeastward from about the center of the Coastal Plain of Texas, including all of Louisiana, into north central Mississippi and dipping southeastward across southwestern Alabama to the Gulf Coast at a point in the western part of Florida. The shape of this anomaly is suggestive of a prehistoric shoreline and of some influence related to the delta-forming history of the Mississippi River.

These two broad patterns of cancer experience (high and low)--and a few others--seem to show exceedingly tempting correlations with certain maps showing broad associations of Great Soils Groups of the United States. The soils maps obviously are related to long range effects of climate and weather on ancient geological formations.

Significance:

1) While it is not understood what immediate practical significance in cancer research can be attached to these observations, it does seem important to know that real geographical differences in the occurrence of neoplastic disease do exist within a human population living and dying on a land mass as large as that of the United States. It is surprising and thought-provoking that broad geophysical and climatic features of the land mass occupied by humans are even suggested by geographic patterns of neoplastic disease based upon a time period as short as five years. Similar demographic observations have proven to be of practical use in elucidation of the cause and control of some other acute and chronic diseases and in other research areas concerned with biological phenomena in general.

2) In anticipation of modern research yielding a more crystallized picture of the mechanisms regulating the growth of living cells and the relation of these to neoplasm, it would appear that maps such as are being produced in this project could serve a useful purpose in tying together and possibly evaluating relative strengths of currently fashionable concepts about the causes of "spontaneous cancer" in human beings.

Proposed Course of the Project:

1) Further refinement of the cancer mortality data will be carried out and plotted, as indicated above.

2) Following a similar methodology, mortality data for cancer of several important sites will be reduced to standardized mortality ratios for counties. In due time it is planned to carry out a similar analysis centered around the census year 1960.

3) One or more papers describing the results of the nationwide analyses are planned for publication. A separate report, either for

publication or for administrative use will be prepared covering the 15-state respiratory cancer analysis.

4) As new information growing out of modern research dealing with earth sciences becomes available, it will be collated and compared with the current cancer pattern maps.

5) New maps showing cancer distribution for different time periods are contemplated and can be easily produced whenever new population and vital statistics become available. Additional working space for preparation, handling and study of large maps would greatly facilitate this work.

Part B Included Yes ☐ No ☒

Serial No. NCI-161
1. Office of Associate Director for Field Studies
2. Epidemiology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Possible Effects of Atomic Fall-out as Reflected in Vital Statistics

Principal Investigator: B. E. Carroll

Other Investigators: P. A. Lawrence

Cooperating Units: None

Man Years (calendar year 1960):
Total: 0
Professional: 0
Other: 0

Project Description:

Objectives: To detect possible harmful effects of fall-out from nuclear weapons testing through their influence on those vital statistics which would be affected by harmful genetic changes in the population or by increased cancer incidence.

Methods Employed:

1) Items of vital statistics which might be affected by direct or by genetic effects of radiation from atomic fall-out have been tabulated for a base-line period of 10 years and for each year following the start of nuclear weapons testing in Nevada.

2) The items tabulated include sex ratio among live births, infant death rates, fetal death ratios, birth rates, death rates from congenital malformations, and death rates from cancer.

3) The tabulations have been made geographically on the basis of the best available estimates of the amount of all-out received in various regions, states, and counties in the search for possible changes along this path which do not occur in other areas. Charts have been constructed to show trends for each variable studied.

Major Findings: No evidence of change has been discovered to date which could not be attributed to chance fluctuations.

Significance:

1) Any increase in cancer mortality along the path of atomic fall-out which did not occur in other areas and not otherwise explained would raise the presumption of increased cancer incidence due to radiation damage.

2) Any unexplained changes in the other items tabulated would raise the presumption of radiation damage to the populations concerned and of possible increases in cancer incidence some years later.

Proposed Course of Project:

1) Data for the base-line years, and through 1955 have been tabulated. As vital statistics become available, data for succeeding years will be added.

2) Results will be presented in reports for administrative use from time to time.

3) The project will need to be continued for a few years in order to have a chance of producing definite results, either positive or negative.

4) Routine reports of atomic fall-out data now being furnished by the Radiological Health Division of PHS will continue to be received and viewed for appropriate comparison with our data.

Part B included ☐ No ☒

PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NCI-154

1. Office of Associate Director for Field Studies
2. Epidemiology Branch
3. Bethesda, Maryland and Chicago, Illinois

Part A.

Project Title: Cancer as a Cause of Death and Disability Among Workers in the Railroad Industry.

Principal Investigator: Benjamin E. Carroll

Other Investigator: Pope A. Lawrence

Cooperating Units: Railroad Retirement Board, National Office of Vital Statistcs

Man Years (calendar year 1960):
Total: 3 1/3
Professional: 1/3
Other: 3

Project Description:

Objectives: 1) To determine occupational and other group differentials in mortality and disability from cancer of various sites (with particular attention to the lung) among employees in the railroad industry.

2) To make comparisons with figures for the general population. Any similarities or differences found can serve as leads for detailed investigations as to specific etiological factors (possibly in cooperation with railroad medical departments.)

Methods Employed: 1) A cohort of all railroad employees with at least 10 years of service, either active in 1951 or retired by the end of 1951, has been set up, numbering about 1,000,000 persons. Data for this cohort covering mortality from all causes and disability retirements due to cancer have been or will be assembled at RRB headquarters from punch-card files and from claims. The desired data are being supplied to the Institute in the Form of duplicate punch cards, tabulations, and claim data representing the defined cohort and the deaths and disabilities occurring among members of the cohort each year. It is expected this cohort will be followed for 10 years. Data will be available to classify members of the cohort and the cancer cases as to age, sex, length of service, occupation and employer for making both gross and detailed comparisons. Since all causes of death are being accounted for, data will be available

of possible interest to other Institutes. The Heart Institute is paying part of the cost of the project, and will be furnished tabulations of data of interest to them.

Major Findings: None as yet.

Significance: Railraod employees constitute an occupationally stable group with their own retirement system. This is an ideal setting in which to attempt investigation of occupational factors and will provide good experience for later handling occupational data collected in Social Security Board operations. Certain railroad employees are known to be exposed to carcinogenic agents, and we have here a direct means of observing the effects. With a defined cohort, holding certain occupational factors constant, it should be possible to supplement the RRB records with collection of additional details on personal histories (in cooperation with individual railroads) to test association with other factors, Considerable interest has recently become manifest on the part of railroad companies and the Association of American Railroads in the outcome of this and other studies on the health of railroad employees.

Proposed Course of the Project: During the first 7 years the operational details of collecting the data from RRB files have been worked out, the cards for the cohort have been selected and tabulated, 6 years' mortality and disability data have been compiled on data sheets, and 4 years' data have been punched into cards. Tabulations have now been planned for the first 3 years' results (1952-1954). On the basis of analysis of these data, to be carried out within the next several months, a preliminary paper will be prepared for publication.

Part B. Included Yes / / No /X/

PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NCI-155
1. Office of Associate Director for Field Studies
2. Epidemiology Branch
3. Cleveland, Ohio and Bethesda, Maryland

Part A.

Project Title: Types of Cancer Among Railroad Engineers, Firemen, and Trainmen.

Principal Investigators: P. A. Lawrence, J. Wm. Lloyd

Other Investigators: B. E. Carroll, D. Smith

Cooperating Units: Brotherhood of Locomotive Firemen and Enginemen, Brotherhood of Railroad Trainmen.

Man Years (calendar year 1960):
Total: 1/2
Professional: Less than 1/3
Other: 1/3

Project Description:

Objectives: Phase 1:

1) To study age and occupational patterns of cancer mortality among a particular segment of railroad employees.

Phase 2:

2) To study certain detailed aspects of malignancies causing deaths among a specific segment of railroad employees, covering detailed site, metastatic sites, if any, histologic type, and method of diagnosis.

3) To compare the findings indicated above with those obtained for other population groups such as those covered by the 10-city and Iowa surveys, or with a matched control group.

Methods Employed: 1) Data have been obtained from the Insurance Department records of the Brotherhood of Railroad Trainmen and the Brotherhood of Locomotive Firemen and Enginemen covering age, length of membership, and local lodge affiliation of all members carrying life insurance, and corresponding data for all insured members dying from 1950 through 1953. These cases are being looked up in death certificate files of NOVS to determine causes of death for analysis according to the variables indicated.

2) Data are being obtained from the Insurance Department records of the Brotherhood of Railroad Trainmen and the Brotherhood of Locomotive Firemen and Enginemen covering pertinent information on a current basis on total insured membership and on all insured members for whom death claims are filed. Each case will be looked up in death certificate files of NOVS and cause of death determined. For each death attributed to cancer, a letter and return report are being sent by the Brotherhood to the attending physician, requesting the pertinent detailed information.

3) Age-adjusted mortality rates for cancer (as well as for other causes) will be computed for each occupational group. In addition, patterns of occurrence of various histologic types of neoplasm will be analyzed in relation to the mortality rates, in addition to the proportions microscopically confirmed, proportions with metastases, etc. for comparison with other population groups as indicated above.

Findings: None as yet. Work is still in progresss on the first phase and data are being collected on a current basis for the second phase, which began on January 1, 1956.

Significance: 1) While this study is, in its first phase, similar to the Railroad Retirement Board study, its real significance lies in the detailed data being obtained in the forward-going study of phase 2.

2) Through the second phase information can be obtained on certain aspects of cancer occurrence in railroad employees which are not available through the Railroad Retirment Board study. The latter is limited to anonymous statistics, while the brotherhoods are assisting us in obtaining data on each case concerning method of diagnosis, histologic type of neoplasm, etc. which will be a valuable supplement to the general mortality data.

Proposed Course of Project: 1) The year 1960 should see the completion of the first phase, namely analysis of the data obtained from existing records. The work has been delayed due to the work load on projects having higher priority, but is now going forward.

2) Since the second phase covers a limited population (approximately 120,000), data covering 3 to 5 years will probably be needed to yield significant results. The lag of a few months in availability of death certificates at NOVS will mean that the project will require 1 to 1 1/2 years to complete after the collection of basic data has been finished.

Part B. Included Yes / / No /X/

PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NCI-150
1. Assoc. Director for Field Studies
2. Epidemiology Branch
3. Pittsburgh, Pennsylvania

Part A.

Project Title: Epidemiology of cancer of the lung, Pittsburgh Epidemiological Laboratory

Principal Investigators: C. D. McClure, M. D., A. G. Gilliam, M. D. and B. K. Milmore, M. D.

Other Investigators: W. McD. Hammon, M. D., and A. Ciocco, Sc. D., Graduate School of Public Health, Univ. of Pittsburgh
K. R. Boucot, M. D., Women's Medical College, Philadelphis, Penn.; George A. Shipman, M. D. U.S.P.H.S., Staten Island, N. Y.; J. William Lloyd, Statistician, U.S.P.H.S., NCI

Cooperating Units: Oncolytic and Oncogenic Section, Dr. Hammon's Virus Laboratory

Man Years (calendar year 1960):

Total: 6
Professional: 4
Other: 2

Project Description:

Objectives:

1) To investigate the clinical and environmental circumstances under which representative cases of cancer of the lung occur in a general population and to determine the characteristics which distinguish people who develop the disease from those who escape it.

2) To partially evaluate the mass small film x-ray survey as a tool for identifying cases.

3) To investigate the application of certain virological techniques in a search for a lung cancer diagnostic tool.

Methods Employed:

1) Personal inverview of reported cases and control population over a period of several years, together with collection of augmentative clinical records from hospitals and physicians of the area.

Part B. included Yes ☐ No ☒

2) Follow-up, through death certification data of the x-rayed and non-x-rayed population; and special and detailed follow-up of selected sample of x-ray examinees who had certain lesions, together with a control group who were negative to x-ray.

3) In cooperation with the oncolytic and oncogenic section of Dr. Hammon's Virus Laboratory, fresh surgical material from human lung tumors is being studied in tissue culture and hetero-transplant systems.

Major Findings:

1) In general study, clinical records and personal interviews are still being collected. Hospital abstracts have been completed on approximately 70 per cent of the nearly 2,500 deaths due to lung cancer occurring in Allegheny County from 1953 through 1958. During this period the trend in lung cancer mortality has gradually risen. The annual rate for white males has increased from 31.7 in 1953 to 41.9 deaths per 100,000 in 1958. Age specific rates are highest among the 70-74 age group ranging from 166.5 in 1953 to 269.3 deaths per 100,000 white males in 1957. Concomitantly, the white male/female ratio has increased from approximately 4.5/1 in 1953 to 10/1 in 1958.

2) In line with data previously presented, the following findings pertain to the evaluation of the mass small film chest x-ray survey as a tool for identifying cases of primary lung cancer.

   a) A 60 month follow-up was carried out on the 1,057 mass survey participants who were identified by Dr. Boucot and/or Dr. Shipman as having "solitary nodules" or other evidence of a lung cancer. Five year data were obtained on more than 93 percent of these subjects showing the following yields of primary lung malignancy. Among those persons with "solitary nodules" identified by the original survey reader the yield was 3.5 per cent, for those with "nodules" identified by Dr. Boucot the yield was 14.2 per cent, and for those with "nodules" identified by Dr. Shipman, 8.3 per cent. Similarly, the respective yields of primary lung cancer among persons suspected of having lung cancer (including solitary nodules) were 8.9 per cent of those suspected by the original survey reader, 20.3 per cent of those suspected by Dr. Boucot, and 14.1 per cent of those suspected by Dr. Shipman. These findings together with similar findings reported previously indicate that small film chest x-ray surveys in selected populations can be more efficiently employed as a tool for identifying unsuspected primary lung cancer.

b) Of the total residents dying of lung cancer during the period 1953-1958, 892 were participants in the mass x-ray survey of 1953. Although most deaths occurred among persons whose survey films were interpreted as "normal" by the original survey reader, the pattern of occurrence within the various reading categories is interesting in that certain categories show high early rates of death due to lung cancer, other show late rising death rates and still other show consistently high rates through out. Further investigation of the clinical and epidemiological significance of these patterns is underway.

3) There are no major findings as yet with regard to the application of virological techniques in search of a lung cancer diagnostic tool since the laboratory methods are still being developed.

Significance to Cancer Research:

1) Provides basic information regarding pathogenesis and epidemiology of cancer of the lung which cannot be determined in any other way.

2) Provides partial evaluation of mass small film x-ray surveys as a lung cancer case finding tool.

3) Provides basic laboratory information regarding lung cancer cases seen in this area.

Proposed Course of Project:

Continued collection and periodic analysis of follow-up data on subjects for a period of several years according to objectives stated above.

PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NCI-151
1. Assoc. Director for Field Studies
2. Epidemiology Branch
3. Baltimore, Maryland

Part A:

Project Title: Baltimore Industry and Lung Cancer Study

Principal Investigator: Frank E. Lundin, Jr., M. D., M.P.H.

Other Investigators: Philip E. Sartwell, M. D., M.P.H., Alexander G. Gilliam, M. D., D.P.H., Patricia B. Geiser, R.N., M.S., (NO)

Cooperating Units: The Johns Hopkins School of Hygiene and Public Health
The Baltimore City Health Department
The Maryland State Health Department
The Bureau of Old Age and Survivors Insurance
All general hospitals in Baltimore and Annapolis

Man Years (calendar year 1960):

Total: 2/3
Professional: 2/3
Other: None

Project Description:

Objectives:

1) To determine Baltimore Metropolitan Area industries which have a high lung cancer risk among their employees.

2) To evaluate certified lung cancer deaths as a measure of lung cancer risk in a population.

Methods Employed:

To achieve the first objective, we will make a case control analysis of industry of employment during the period 1937-1955 among lung cancer deaths and controls. The "cases" are all 1954 through 1956 male lung cancer deaths in the Baltimore metropolitan area residents who had had recent social security covered employment. The controls are a race and age matched probability sample of working social security covered males in the area in 1955. BOASI will provide the employment histories on an estimated 500 deaths and 500 controls.

Part B. included Yes ☐ No ☒

To achieve the second objective, we are doing a 1955 hospital morbidity survey of newly diagnosed lung cancer cases. We will compare abstracts of the hospital records of expired cases with their death certificates. We will also trace a sample of lung cancer death certificates and determine their hospital diagnoses.

Major Findings:

There are no findings to date. Please see proposed course of project.

Significance to Biomedical Research and the Program of the Institute:

This study should allow us to:

1) Provide information regarding high lung cancer risk in industries.
2) Provide an estimate of the validity of using lung cancer death certificates in epidemiological studies.
3) Provide an evaluation of BOASI information as far as its usefulness in disease study is concerned.
4) Make contributions to methodology of studying industrial groups.

Proposed Course of Project:

The 1955 morbidity survey has progressed to the fifth of twenty-two hospitals in the area. Negotiations with both health departments have been made for the acquisition of death certificate information. BOASI negotiations are now at a stage where a letter is being drafted by Mr. Charles Potter of BOASI to be sent to the Commissioner for special authorization. We plan to have the BOASI data before July 1, 1961 and to analyze the data after that time.

PHS-NIH
Individual Project Report
Calendar Year 1960

NCI-
Serial No. 156
1. Office of Associate Director for Field Studies
2. Epidemiology Branch
3. Hagerstown, Maryland

Part A.

Project Title: Geographic and Environmental Factors in Cancer Mortality in Washington County, Maryland

Principal Investigators: P. A. Lawrence, Dr. W. Y. Chen, Dr. L. B. Crittenden

Other Investigators: B. E. Carroll, H. Greenville, C. Pinkerton, R. Bogdanoff, Dr. R. Cameron, Dr. V. E. Archer

Cooperating Units: Washington County Health Department, State of Maryland; U. S. Geological Survey

Man Years (calendar year 1960):		1961 Estimate
Total:	22 1/2	17
Professional:	7 1/2	6
Other:	15	11

Project Description:

Objectives: 1) To determine geographic patterns of cancer mortality and trends for a period of several decades in one American county.

2) To relate geographic patterns of cancer mortality to measurable variations in environmental factors in search of clues toward the etiology of "spontaneous" human cancer.

3) To develop and test methods for application on an enlarged scale to the problem of explanation of known geographical differences in cancer occurrence within the United States.

Methods Employed: 1) House-to-house interview, collecting personal data on each occupant, and obtaining information on many environmental factors. The interview information is supplemented by obtaining information relative to all individuals who have lived in the house for the last ten years. Death certificates and hospital records are used for obtaining and checking cancer data. Old census records, newspapers files, power company records, etc. are used to complete the epidemiological data.

2) Extraction of environmental information from available maps and records relative to each house. This includes approximately fifty different items, including such items as distance from roads, streams, other houses, physiography, drainage, soil types, geology, vegetation,

etc. The sources of these items include geological maps, tax maps, highway maps, soil maps, airphoto maps and isoradioactivity contour maps. For the initial analysis, less than half these data will be coded.

3) Detailed measurements of alpha and beta activity are being made on samples of soil, water and potatoes from gardens of selected Index (house where someone developed cancer) and Non-Index Houses. The water is also being analyzed for detergent content as an index for surface pollution.

4) A geohydrologic survey, including a piezometric map of underground water supply, has been completed in one unusually interesting community.

5) The data are being coded and put on IBM punch cards for analysis by life table techniques. Some of the data may be unsuitable for analysis in this way and so will have to be done by hand or by keysort cards.

6) Death certificates have been collected on married couples and siblings who have died of cancer. These are being analyzed to see if the frequency of type of cancer is greater than would occur by chance.

Major Findings: 1) There are statistically significant differences in cancer mortality rates between several of the election districts in Washington County. This is based on data adjusted for age.

2) For 7,490 single residence houses in the city of Hagerstown there was a greater number that had 2 or 3 cancer deaths than would be expected purely by chance. However, such items as age of houses or selective population movements could possibly account for these differences.

3) A house-to-house scintillometer survey has confirmed our impressions than the airborne gamma survey gives an excellent average background figure for sizable areas or groups of houses, but that for any analysis requiring pairing of houses or accurate data on individual houses it is inadequate and must be supplemented by ground measurements.

4) There is extensive poliution of the well waters in one town having high cancer mortality. This was shown by detergent analysis and coliform culture done on all wells in or near the town. Water in this community also shows a peculiarly high nitrate content.

5) A preliminary geochemical study of rocks, soils and plants in Washington County seems to point toward interesting variations in the occurrence of such biologically significant elements as manganese,

molybdenum, zinc, copper, chromium, titanium and lead.

6) Monthly radiometric analysis of water from 16 sources showed considerable variation during the year. A single sample would be subject to considerable error if it was considered to be representative.

Significance: The idea that human cancer may be related to transmissible or variable environmental factors recurs persistently in world literature and folklore. Notions about such things as "cancer houses" and "cancer families" have enjoyed periodic bursts of fashionable interest, yet until recent years, very little actual field work seems to have been done to develop unbiased evidence to establish the existence of such things. Washington County, Maryland provides unique opportunities to assemble facts in considerable detail about distribution of cancer among a fairly stable population group. These, combined with modern scientific methods for collecting, and processing for analysis, large volumes of appropriate environmental data afford new opportunities for evaluating this old idea. This project may contribute significantly new knowledge and at the least provide a human proving ground with which to test new concepts about etiology, and maybe even prevention, of cancer, as they emerge from the intensive and far-flung bench laboratory researches currently in progress.

Proposed Course of the Project: 1) Completion of the ten year population data, soil and geological data for 17 election districts on a district by district basis, leaving Williamsport, Conococheague, Funkstown, Clear Spring and Wilson to the last as they have the most work yet to be done. Complete as many as possible by June, 1961. The first three are now completed.

2) Life table analysis of the above data in various steps, starting with the three election districts already completed, adding other districts as they are completed.

3) Complete the revised analysis of 30 year mortality rates by election district before June 1961.

4) Complete several miscellaneous analyses, such as the cancer site distribution in 150 husbands and wives, and the analysis of environmental data relating to approximately 150 houses in the Index vs. Non-Index House Study (Index houses being ones in which cancers have developed, and Non-Index in which no record of cancer development is known).

5) Evaluation of the micro-epidemiological analyses mentioned above is anticipated around the first of July 1961 at which time the future direction of environmental field activities will be determined.

PHS-NIH
INDIVIDUAL PROJECT REPORT
Calendar Year 1960

Serial No. NCI-157
1. Office of Associate Director for Field Studies
2. Epidemiology Branch
3. Salt Lake City, Utah

Part A.

Project Title: Study of the Cancer Experience of Non-Uranium Hard Rock Miners in the Rocky Mountain Area

Principal Investigators: V. E. Archer, M.D., and M. Erjavec Haij, R.N.

Other Investigators: P. A. Lawrence, B. E. Carroll, D. A. Holaday

Cooperating Units: Certain mining companies, and the Salt Lake City Occupational Health Field Station, DOH, Bureau of State Services, PHS.

Man Years (Calendar year 1960):		1961 Estimate:
Total	1/8	1/8
Professional	1/8	1/8
Other	0	0

Project Description:

Objectives: 1) To evaluate the prevalence of cancer of the lung and other diseases among underground non-uranium miners in the Rocky Mountain area.

2) To answer the question: Do hard rock miners (other than uranium) have a lung cancer rate higher than normal? If so, is there a relationship to radon in the mines?

3) To have these data from non-uranium mines for comparison with the results of the Uranium Miner Study.

Methods Employed: 1) Assembly of occupational, medical and vital statistics information on a composite group of hard rock miners with at least 15 years of underground working experience in non-uranium mines in the Rocky Mountain States.

2) Collation and medical statistical analysis of the collected data with particular emphasis on lung cancer cases and deaths that may occur; calculation of "expected" rates.

3) Making measurements of radon in the mines in which the study group worked. These measurements must be correlated with ventilation and projected backward 30-40 years.

Major Findings: None to date. We have collected data on a study group of 1766 men who completed a minimum of 15 years underground at some time between 1937 and 1948. There have been 870 known deaths in this group. Life tables have been prepared. The radon and radon daughter levels in the subject mines have been measured. They have not yet been correlated with ventilation changes and projected back 30 years.

Significance: 1) Data on human beings exposed to a potentially carcinogenic radioactive environment will be compiled and so become available for possible use in cancer control. At present, such information is extremely scarce. Limited studies of mining communities in Europe have revealed a high incidence of lung cancer in miners working in mines containing radioactive dust and gas. Extensive animal experiments in Europe and the Americas as well as observations made on human beings inadvertently exposed to excessive radiation over prolonged periods of time have established beyond doubt that ionizing radiation causes cancers in animals and in people.

2) In addition, this study may yield not only information regarding levels of radiation which are harmful, but also levels of radiation in which miners may work and suffer no harmful effects. This information would be of value in establishing recommendations for the uranium mining industry.

Proposed Course of Project: 1) Complete statistical analysis. Work is going forward at present on computation of appropriate mortality rates for comparison with those of the hard-rock miners. A manuscript will be ready for publication within the next few months comparing mortality experience of this group with that of the general male population and with that of the uranium miners.

2) Complete "backward estimates" of radon levels in the mines involved.

3) Add above information to the present draft of manuscript and present to the companies involved with a request for release to be published.

Part B. Included Yes / / No / X /

PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NCI-158
1. Office of Associate Director for Field Studies
2. Epidemiology Branch
3. Salt Lake City, Utah

Part A.

Project Title: Cancer Occurrence in Uranium Miners of the Colorado Plateau

Principal Investigators: Victor E. Archer, M.D., and P. A. Lawrence

Other Investigators: B. E. Carroll, J. K. Wagoner, Clayton Phelan, D. A. Holaday, Marylou Ingram, M.D., A. W. Hilberg, M.D.

Cooperating Units: Salt Lake City Field Station of the Division of Occupational Health; Grand Junction Operations Office of the Atomic Energy Commission; the University of Rochester School of Medicine & Dentistry; The Indian Health Service; the State Health Departments of Utah, Colorado, and New Mexico. Division of Radiological Health, BSS, PHS.

Man Years	(Calendar Year 1960):	1961 Estimate:
Total	9	4
Professional	2.5	2
Other	6.5	2

Project Description:

Objectives: To obtain complete or partial answers to the following questions:

1) Does lung cancer occur more frequently among uranium miners than amont other miners or among other males of the Rocky Mountain states?

2) If "yes" to question one, can the excess lung cancer be attributed to radiation from radon and its daughter products?

3) Are any other diseases more prevalent among uranium miners than other men?

4) What early signs can be detected which will indicate a radiation effect in uranium miners prior to development of lung cancer?

5) Is the currently accepted "working level" of radioactive dust and gas a safe level to permit men to work in continuously?

Methods Employed:

1. Triennial physical examinations are done on as many uranium miners as possible who work in the Colorado Plateau Area. Detailed identifying data are obtained and a standard physical examination including chest X-ray, blood and urine tests is done. These examinations were made on 1788 uranium miners in 1960, bringing the total number of miners examined to approximately 5500.

In addition, special tests which may indicate radiation effects are done. In 1960, this included Timed Vital Capacity, differential white blood count, Binucleate Lymphocyte Count, Sputum Cytology and Finger Nail Cystine.

2. An extensive follow-up program including an annual mine-to-mine census has been developed to maintain contact with each uranium miner and to determine causes of death in the group.

3. The data collected from the medical history and examination are analyzed by multiple and partial correlation and other techniques to determine any correlations with age, altitude, job, years of underground mining or the level of radiation under which the miners work.

4. The mortality data are analyzed by the life-table method and compared to other mining groups (not examined) and to the general male population.

5. Frequent measurements of radon daughter concentrations in the uranium mines are made by industrial hygiene engineers in several of the cooperating groups named above.

6. Searching for lung cancer deaths among uranium miners in the past 20 years, who were not included in our study. This is done by: 1) Obtaining death certificates mentioning lung cancer in males in selected counties of Utah and Colorado, and 2) Making local inquiry to determine occupational experience of these men in uranium mines.

Estimates of the number and age distribution of uranium miners in early years of the industry provide a population base for judging the overall significance of the lung cancer experience of the early uranium miner population.

Major Findings:

1. A life table analysis has been completed in which the mortality experience of uranium miners in the study can be compared with that of other groups of men. The three major findings of this was that non-motor vehicle accidents was high among uranium miners, that cor pulmonale was important as a cause of death, and that lung cancer was

significantly high in the men who had mined uranium underground for three years or longer. The latter observation, however, is based upon only five cases of lung cancer.

2. The 1960 sputum cytology studies have not been completed, but of 272 specimens examined, there were 33 (12%) suspicious, and 9 (3.3%) positive.

3. Preliminary correlation analyses indicate that years underground, job or radiation level in mines affects the incidence of suspicious sputums, pulmonary function, hematocrit, leukocyte count, basophils, neutrophils, monocytes, eosinophils, lymphocytes, binucleated lymphocytes and dysplasia of hair roots.

Significance:

1. During the latter part of the last century and early part of this century, death rates from lung cancer in uranium miners in Europe were in the range of 50-75% of all causes of death. There is reason to believe, because of high concentrations of radio-active gas and dust in mines in this country, that a similar hazard could exist and it is believed necessary to evaluate the possible risk.

2. This study may well produce results which will help in setting maximum permissible radiation exposure levels in other occupations and to the general population.

3. This study may for the first time demonstrate subclinical biological effects of radiation in uranium miners. These changes may be of value as indices of exposure to certain kinds of radiation. If found to be correlated with later diagnosis of cancer. these subtle changes might even prove useful in early detection of the disease.

Future Course of the Project:

There is a tacit understanding growing out of the recent reorganization of PHS that the kind of field research represented by this project fits more properly into the philosophical framework of the anticipated new Bureau of Environmental Health. Responsibility for conduct of future field activities on this project, therefore, passes from NCI. There is agreement, however, that NCI's full responsibility for work done over the past seven years will have been properly discharged only by:

1) Compilation and analysis of all medical, mortality and follow-up data collected under NCI direction through the 1960 field physical examination program which ended in September 1960.

2) Conclusion of present NCI contracts with outside medical experts in the fields of Hematology, Pathology and Sputum Cytology.

3) Publication of results. A preliminary paper has already been prepared, others are in the offing, and during 1961 intensive effort will be expended by the Branch staff to accomplish all 3 of the objectives set forth above.

Patient Material:

Efforts are made to include all uranium miners working in the Colorado Plateau area in this study, and to date over 5,000 men have been examined.

Part B. Included Yes [ ] No [X]

PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NCI-159
1. Office of Associate Director for Field Studies
2. Epidemiology Branch
3. Salt Lake City, Utah

Part A.

Project Title: Study of Occupational Backgrounds of Hospitalized Cases of lung and Bowel Cancer, Leukemias, and Primary Malignant Tumors of Bone in Males Over the Age of 25 Years.

Principal Investigators: V. E. Archer, M.D., and Marian Erjavec Haij, R.N.

Other Investigators: B. E. Carroll

Cooperating Units: Salt Lake General Hospital; two Salt Lake VA Hospitals; LDS Hospital in Salt Lake City, and St. Mark's in Salt Lake City; Dee and St. Benedicts Hospital in Ogden; Utah Valley Hospital, Provo; VA Hospital in Grand Junction; St. Mary's and Holy Cross Hospital in Salt Lake City.

Man Years (calendar year 1960):		1961 Estimate:
Total:	1/8	1/4
Professional:	1/8	1/4
Other:	0	0

Project Description:

Objectives: 1) To determine what occupational backgrounds are found in cases of the above-mentioned malignancies in order to focus attention on any occupations which by reason of frequent listing might be suspected of predisposing to the specific malignancy.

2) To gather data on possible environmental carcinogens.

Methods Employed: 1) Selected internes or residents in the cooperating hospitals are paid four dollars for each detailed history they obtain from patients in the designated categories.

2) Data on all the cases are analyzed biostatistically in search of factors common to the personal and working histories of the patients.

Major Findings: No significant findings to date. Within the year (1960) histories were collected on 47 new cases, bringing the cumulative total up to 421. This body of data consists of 187 cases of cancer of the lung, 87 of leukemia and lymphoma, and 147 of cancer

of large bowel.

Significance: It is hoped that this study will give useful leads for a more detailed study of the environmental factors in some cancers.

Proposed Course of Project: 1) Continued accrual of cases until larger numbers have been collected.

2) Biostatistical analysis.

Patient Material: Most cases of the designated cancers seen in the major hospitals in Utah and Western Colorado.

Part B. Included. Yes [ ] No [X]

Serial No. NCI-152

1. Office of Associate Director for Field Studies
2. Epidemiology Branch
3. Boston, Massachusetts

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies of Etiological Factors in Leukemia and Other Malignancies of Childhood.

Principal Investigators: Dr. Miriam D. Manning and B. E. Carroll

Other Investigators: P. A. Lawrence

Cooperating Units: Children's Cancer Research Foundation; Allergy Department, Children's Hospital Medical Center, Boston; X-Ray Department, Children's Hospital Medical Center, Boston; Ear, Nose and Throat Department, Children's Hospital Medical Center; Professor of Epidemiology, Harvard, Johns Hopkins and Minnesota Schools of Public Health; Study Directors in 14 national centers for cooperative study and their patient material.

Man Years (calendar year 1960):

Total:	3 1/4
Professional:	2 1/4
Other:	1

Project Description:

Objectives:

To determine etiologic factors and co-factors in Acute Leukemia of Childhood.

Methods Employed:

1) Interviewing mothers of children with acute leukemia and other malignancies using standard questionnaire previously developed.

2) Skin testing of children with acute leukemia and other malignancies for 36 inhalant and 36 food allergens.

3) Coordinating a National Cooperative Child Health (Leukemic) Study which is gathering data by the interview method from 14 centers; also contributing New England cases.

4) Followup studies on 480 children previously irradiated for lymphoid hyperplasia.

5) Obtaining death certificate data in New England on leukemia of all ages for correlation of rates with possible environmental etiologic factors.

Major Findings

1) In 401 cases of acute leukemia of childhood, antenatal X-ray is significantly higher in leukemic children as compared with children with other malignancies.

2) A significantly higher number of mothers of 401 leukemic children have a positive history of hay fever, asthma or hives--this also is true for the leukemic children themselves to a slightly lesser degree.

3) No correlation has been found between previous therapeutic irradiation for lymphoid hyperplasia and the development of leukemia or thyroid cancer (a study of 481 children irradiated).

4) There are counties in Massachusetts, New Hampshire and Vermont where important differences in leukemic rates for all ages exist.

Significance to Cancer Research

1) These studies help clarify the relation of ionizing irradiation to Leukemia.

2) Suggest leads for further and more definitive studies on the relation of hypersensitivity and leukemia.

3) Locate areas of "outbreaks" of leukemia which can be studied in detail.

4) Bring together cooperating institutions who are already organized to carry on further studies.

Proposed Course of Studies

1) To continue interviews in the Boston area until approximately 500 mothers of leukemic children have been interviewed with a corresponding number of mothers of children with other malignancies.

2) To assemble and analyze data on geographic location of leukemic "outbreaks" or consistently higher rates and do detailed environmental studies in these areas.

3) To collect and analyze the data from the National Cooperative Leukemia Study and, using leads gained, plan further research. Data collection expected to be finished by September 30, 1961.

4) To continue skin testing of children at the Children's Cancer Research Foundation until a sufficient number for study has been done.

5) Finish the paper (with the X-ray Department) on Children Irradiated for Lymphoid Hyperplasia.

Part B included Yes ☐ No ☒

PHS-NIH
Individual Project Report
Calendar Year 1960

Serial No. NCI-160
1. Office of Associate Director for Field Studies
2. Epidemiology Branch
3. Boston, Massachusetts

Project A.

Project Title: Cancer Mortality among Catholic Brothers and Sisters in Several Teaching Orders in the United States

Principal Investigators: B. E. Carroll and J. W. Lloyd

Other Investigators: B. K. Milmore, M. D. and William Haenszel

Cooperating Units: University of North Carolina (Institute for Research and Social Science).

Man Years (calendar year 1960):

Total:	2/3
Professional	1/3
Other:	1/3

Project Description:

Objectives:

1) To determine the mortality in some of the larger Catholic teaching orders for all causes and particularly mortality attributed to specific cancer sites.

2) To compare the risk of dying of cancer of specific sites with the corresponding risk in the general population to determine whether significant excesses or deficits exist for any cancer site.

Methods Employed:

1) Information on approximately 70,000 individuals comprising 62 Catholic religious communities from 1900 to 1954 has been gathered, coded and analyzed by means of IBM punch cards in another study carried out in the Institute for Research and Social Science of the University of North Carolina. Although the fact of death was recorded and punched, no attempt was made to determine cause.

2) Through a contract with the above-named Institute the identifying data on all deceased individuals has been duplicated and a deck of IBM cards on the entire population under study has been furnished.

3) The causes of death are being obtained through appropriate channels from the death certificate of each deceased person.

4) Appropriate tabulation and biometric analysis is to be made of the mortality data to discover similarities to and differences from similar data relative to the general population.

Major Findings: None. Data are now being collected.

Significance to Cancer Research: This study will complement a similar research project recently completed by Dr. Taylor, Mr. Carroll, and Mr. Lloyd. It is much larger in scope and should help clarify some fragmentary evidence indicating that incidence of certain forms of cancer is different for members of some religious orders than the general population, possibly due to difference in manner of living. It may make possible a significant contribution to our knowledge of cancer.

Proposed Course of Project: The National Office of Vital Statistics will begin coding causes of death by February 1, 1961. This phase of the work should be completed by March 15. Finished punch cards will be ready for machine processing by April 15. Life table analysis will follow as quickly as machine programming time allows.

Part B. included Yes / / No /X/

I. Introduction

This report describes developments in the extramural grant fellowship and training areas of the National Cancer Institute program during the calendar year 1960, the status and significant achievements of continuing activities for this period, and administrative and scientific program planning for the future.

II. Developments of Special Interest

The National Cancer Institute program of support for medical research and research training is expanding and becoming more diverse in character. New programs implemented this year and to be developed in the near future have required the formulation of grant policies which assist not only the individual investigator but the scientific research institution as a whole. Consequently there is a correlated need to develop manpower resources and to strengthen physical plants, housing, health research and education.

An overall policy statement of the National Institutes of Health covering Institutional Grants was issued November 8, 1960. The purpose of institutional grants is to provide funds for the general support of medical research and research training programs of institutions under conditions enabling recipients to meet research and research training needs not adequately met by existing modes of support. Schools of medicine, osteopathy, public health and dentistry will be eligible to apply for such grants in the fiscal year ending June 30, 1961. These institutional grants will enable the recipients to operate cancer research programs with additional flexibility in programming for the support of current research, salaries, ancillary research services (shops, animals, instrumentation, research publications, etc.), renovation and repair of research facilities, and the like. A reserve of about $2,000,000 has been allocated during the fiscal year 1961 for these grants.

A program for the development of Clinical Cancer Research Centers has been started. These clinical research centers will provide a continuing framework for clinical research effort, including the necessary laboratory, clinical and supporting services. The goal of a clinical research center is to establish a stable organizational framework, an atmosphere of common purpose, and an intellectual and scientific environment for integration and concentration of clinical investigation, with concomitant exacting observations on patients.

The National Cancer Institute began development of a program to award Cancer Research Professorship Grants to appropriate institutions, to establish additional full professorial positions in scientific disciplines concerned with cancer research. Initally granted for 5 years, these are potentially long-term awards, and will assist in recruiting for research

positions persons with the most thorough training, and the greatest aptitude and promise. The grants would provide full-time, 12 month salaries. In fiscal year 1961 $300,000 will be made available for these grants.

The Senior Fellowship program will provide support for approximately two hundred research and teaching positions in preclinical science departments throughout the country. These Senior Fellowships are intended (1) to encourage and support the development of professional personnel for academic and research careers in cancer medicine and related areas, and (2) aid in stablizing careers in such fields. In fiscal year 1961, funds have been appropriated to permit support of this program and that of the Special Fellowships Grant Program for a combined total of $100,000.

The Special Fellowship Grant Program* is an entirely new mechanism for the development and support of young independent investigators in clinical, preclinical and premedical areas and fields of cancer research to begin in 1961. Individuals eligible for Special Fellowships fall into three groups: (1) the worker who has had at least 3 years of relevant research and/or professional experience after receipt of the doctoral degree; (2) the individual who has completed the residency training requirements in a clinical specialty or its equivalent and (3) the individual who does not have a doctoral or equivalent degree, but who needs special training not met by other fellowship programs. Such grants are intended to provide advanced or specialized training to further the development of a research and academic career in medicine, dentistry, public health and related areas. The grant will support the young investigator not yet eligible for an award at a higher level, for periods of 3 to 5 years. The policy rules prescribed for research grants govern the administration and operation of these grants.

III. Organization and Management

During 1960 the Surgeon General approved a plan for reorganization of the National Cancer Institute, which among other things would consolidate all grants and extramural training activities of the Institute under an Associate Director for Grants and Training. We are now implementing the new plan by the creation of three branches (Research Grants, Training and Operations) to meet the needs of a dynamic program. Three new scientific programs assigned to the Associate Director: Virus Activities, Cancer Clinical Research Centers, and Research Facilities Construction, will be housed organizationally in the Associate Director's office.

The Area completed in 1960 plans for a training program with the objective of developing competence to deal with the many administrative and technical problems arising in the management of grants programs. To accomplish this objective, young people of high caliber are to be selected from the Federal Service Entrance Examination Register, to train with administrative and scientific personnel in the development of specific

*Not to be confused with the Cancer Special Fellowships Awards Program.

program details. This will enable the ranking scientific staff to concentrate on the larger policy aspects of expanding programs. The advantages expected are (1) the high development of applied, specialized on the job skills which colleges ordinarily do not provide, and (2) the performance of many kinds of scientific work at appropriate classified grade levels. The first two appointees under this training plan are scheduled to report for duty January 3, 1961.

IV. Current Programs

The Research Grants Program

Cancer research grants are awarded to scientific institutions, in the name and for the specific support of qualified applicants, to conduct research projects on some aspect of, for example, (1) the causes, epidemiology, nature and therapy of cancer and (2) basic research related to the cancer problem.

Steadily increasing progress towards the ultimate goal of understanding and eradicating human cancer can be reported as a definitely encouraging result of cancer grant research accomplished in 1960. Stimulating scientific papers were published in major experimental program areas supported by grant fund, including those dealing with

(a) cancer causation
(b) morphology of cancer development
(c) physiological chemistry of cancer
(d) host-tumor relations
(e) diagnosis and associated techniques
(f) epidemiology
(g) therapy of cancer

Examples of research accomplished under the Research Grants Program follow:

Cancer caused by internal factors of the environment

Arsenic compounds are dispersed as contaminents in some industrial atmospheres, and have been implicated as a cause of lung cancer by Dr. Robert H. Holland and his co-workers of the University of Texas Southwestern Medical School. Arsenic has been reported also in filter cigarettes in amounts these scientists regard as dangerous. They examined fifteen lungs of cancer victims from industrial localities, and 23 lungs from non-cancer patients from other localities: the average amount of arsenic in cancerous lung tissues (7.14 milligrams per gram of tissue) was significantly higher than that in the control group (4.13 milligrams). Dr. Holland and his group likewise observed that there has been a 200 to 600% increase in the amount of arsenic detectable in cigarettes in the past 25 years. They tested seven leading brands of filter cigarettes for arsenic content of the cigarette and filter prior to smoking, and the filter, butt and ash after smoking. Filter cigarettes appeared to contain more arsenic than regular cigarettes, and in such quantities that they believe a probable causal relationship does exist between the arsenic inhaled in cigarette smoke

and the development of lung cancer. [1]

The chromate dusts dispersed in the air incident to the processing of chromate ores and the manufacture of chromium compounds have been under suspicion as a cause of lung cancer. During the last year Dr. A. M. Baetjer and his associates of The Johns Hopkins University have investigated the possible role of chromium compounds in the development of bronchial cancer. Analyses they made of lung tissue from workers in chromate processing plants and taken at autopsy, indicated that lung cancer could not be related directly to the concentration of chromium in the lung tissue. To test their observations further, they also studied the effect of chromium compounds on the development of lung tumors in mice and rats. In order to reproduce as nearly as possible the conditions existing in chromate processing, these laboratory animals inhaled chromate dusts in a carefully controlled experimental environment. In another test various finely divided chromates were introduced directly into their tracheae and bronchi under anesthesia. A third group of animals received zinc chromate or barium chromate by injection into the tail vein: and a fourth lot had mixed chromate dust injected into the pleural cavity. It was found that the animals exposed to the chromate dust suspensions in the experimental air chamber did not develop lung cancer, a result similarly found to be true of animals injected intratracheally and intraperitoneally. [2]

Many different chemical compounds may induce experimental cancer, and much effort has been devoted to search for a characteristic molecular structural pattern which could be uniquely associated with the mechanics of cancer development. No single molecular pattern has been found thus far. In the aromatic class of organic compounds where three benzene rings are linked in the phenanthrene configuration, one of the carbon-to-carbon valence linkages occupies a position thought to be significant in producing a cancer-causing agent. Drs. Odilon Chalvet and Raymond Daudel of the Centre de Chimie Theoreque de France and Dr. Carl Moser of the Pasteur Institute have investigated the energies required to bind elements in molecules and the relations of the electrons involved. One of the effects is to increase the charge, and when this happens, there is a greater tendency for the substance to be a cancer-causing agent. Eight compounds having the highest levels of energy evaluated were all strong cancer inducers and there was in each case a carbon-to-carbon linkage in the same molecular region, termed the "K" region.[3] Dr. E. J. Mariconi and his associates of Fordam University have attached the kinetically powerful oxygen derivative, ozone, to the "K" region of phenanthrene, and also to a similar "L" region in 1:2-benzanthracene. These scientists believe that, in a series of related compounds of which anthracene, 1:2-benzathracene and dibenzathracene were members, this ozonolysis reaction demonstrated a significant correlation between cancer-causing potency and chemical reactivity at the "K and "L" regions.[4]

The possibility that cancer may be induced by x-radiation used for diagnostic purposes in the detection of cancer, or for treatment of this disease, is of growing concern. Drs. E. L. Saenger and his colleagues of the University of Cincinnati and the Cincinnati Children's Hospital have continously scrutinized the collateral effects of therapeutic irradiation

for cancer in children since 1956. One thousand, six hundred and forty-four persons who had been irradiated over the head, neck and chest in infancy and childhood were compared with 3,777 of their brothers and sisters with respect to their subsequent medical history. Eleven cancers of the thyroid gland were found in the patient group, but none were found in the normal control group. These workers consider that the incidence of cancer of the thyroid in the irradiated group is about 100 times that expected from current tables of morbidity. Likewise, the incidence of infectious and other non-cancerous illness was greater among the patients than among their brothers and sisters. Nevertheless Dr. Saenger is of the opinion that radiation does not appear to be the sole factor responsible for this increased incidence, but is rather a contributing factor. It is recommended that minimal radiation doses over the smallest practicable areas be employed with proper shielding, when this therapy is the one of choice. 5

The circumstances surrounding the radiation induction of thyroid cancer have been investigated also by Drs. Nechama Haran-Ghera, Jacob Furth, Rita F. Buffett and Kenjiro Yokoro of the Children's Cancer Research Foundation. They evaluated the effects of radiation from atomic detonation and from x-radiation on laboratory mice. In animals which were completely exposed to atomic radiation, thyroid tumors were caused in 3.4 to 7.3%. No thyroid tumors were found in the x-irradiated mice. In atom-irradiated mice from which the adrenal glands had been removed, an icidence of 1.6 to 2.9% was reported for thyroid tumors; when the animals were castrated, the incidence was 2.5%. These investigators suggest that the stresses caused by the removal of these glands facilitated thyroid tumor development. Some tumors of the adrenal gland were produced when mice were exposed to atomic detonation, and the incidence ranged from 2.7 to 3.6% among the several unit groups so irradiated. It was concluded that atomic radiation had a higher effectiveness in relation to x-rays for thyroid and adrenal tumor induction. 6

Radioactive cobalt-60 emits the most penetrating, "hard" type of atomic radiation, the gamma ray. Drs. Olive Gates and Shields Warren of the New England Deaconess Hospital have found lung cancers to develop in mice exposed to this form of radiation. The metal was implanted in the right lung or pleural cavity in the form of wire about 2 mm. by 0.5 mm. Eleven out of 13 animals survived beyond 11 days, and 4 beyond 100 days. In the latter, the shortest elapsed time interval after which lung cancer could be found was 97 days. In another group of mice, 20 of 190 cobalt-60 treated mice which lived 97 or more days developed cancer of the lung or bronchus. 7

Recent studies on the relationships of viruses to the induction of tumors have developed new knowledge concerning the appearance, nature and behavior of viruses in the cell. Grant-supported research during the year was devoted in large measure to the causal relations between viruses and leukemia, mammary tumors, salivary gland cancer, polyoma, and the Rous sarcoma. Technological advances in laboratory methods have been made with the use of newborn animals, electron microscopy of ultrathin tumor sections, and tissue culture.

Dr. Leon Dmochowski and his colleagues of the University of Texas M. D. Anderson Hospital and Tumor Institute and the Baylor University College of Medicine have undertaken a study of the relationship of viruses to the origin of the human white blood cell type of cancer, leukemia. Electron microscope studies have been carried out on ultrathin section of lymph nodes taken from 6 patients with various forms of leukemia. Virus particles approximately 900 angstroms in diameter have been observed in cells from 3 cases of acute lymphatic and 1 case of acute myeloid leukemia, and 1 of lymphosarcoma. There were no particles in non-leukemic control tissues. Cell-free extracts of lymph nodes from 8 patients tested have induced changes in monkey kidney cell cultures which could be serially transmitted; such changes were not seen in cancer-free cultures treated with extract of a non-leukemic lymph node. It is of particular interest that in one patient with the white cell cancer lymphosarcoma, not only were virus particles observed in ultrathin biopsy section, but similar particles have been found in cells from the same biopsy specimen grown in tissue culture for six sequential passages during which evidence of change due to the disease had been observed. [8]

In another research project Dr. Dmochowski and his co-workers have sought to characterize any submicroscopic changes and specific structures found in tissues infected with polyoma virus and in tumors considered to be induced by the agent. Mouse embryo tissues of several strains were cultured and changes attributable to the virus have been observed regularly within 4 to 7 days in embryonic tissues of some strains. Spherical particles 270 angstroms in diameter have been observed in the affected cell, chiefly in the nucleus. In some particles an internal structure could be discerned, composed of a denser center 50 angstroms in diameter, surrounded by an electron-translucent outer zone outlined by a limiting membrane. Particles of this type have not been observed in uninfected control cultures.[9]

In research complementing that performed at the M. D. Anderson Hospital and Tumor Institute on viruses, Dr. Charlotte Friend and her associates of the Sloan-Kettering Institute for Cancer Research have investigated the electron-microscopic pictures of particles found in mouse tissues associated with the leukemic agent. In collaboration with Dr. Etienne de Harven of the Université Libre de Bruxelles (Brussels), she observed that individual particles of the mouse type of leukemia virus have an external diameter averaging 870 angstroms, and they appear to be formed at the cell membranes by a budding process. The cells examined were megakaryocytes, and they were treated with lead hydroxide for observation as they appear to have a considerable affinity for this compound. [10] Dr. Moore with Dr. B. E. Eddy and others, have also explored the biochemical reactions of the SE polyoma virus held to cause the cancer-like polyoma of mice. It had been believed that the essential viral component was ribonucleic acid, but the present investigators show that exposure of the nucleic acid preparation to equivalent concentrations of the enzyme deoxyribonuclease for an optimum time completely destroyed the infectivity. It appears then, that the fundamental structure of the SE polyoma virus is deoxyribonucleic acid. [11]

Cell-free filtrates prepared from the brains of patients who have died of leukemia have been shown to accelerate the development of this type of cancer in a highly susceptible (AKR) strain of mice. Dr. Stephen O. Schwartz and his associates of the Hektoen Institute for Medical Research have noted that this filtrate, which behaves as though a viral agent were present, will induce leukemia in about 15% of the inoculated mice when it is concentrated or its rate of absorption increased. When the enzyme hyaluronidase was added to the filtrate leukemia appeared in nearly 50% of the mice. [12]

Drs. George A. Edwards and Rita F. Buffett of the New York State Department of Health, working as scientific associates with Dr. Jacob Furth of the Roswell Park Memorial Institute, have studied a salivary gland tumor from a mouse infected at birth with the polyoma virus, using electron microscopy. Particles were observed both in the nuclei and the surrounding cell field, and likewise between cells. Particles inside the nuclei averaged 30 millimicra in diameter and appeared to be associated with fine filamentous material. Other presumed viral particles of larger size (80 millimicra) suggestive of those seen in leukemia, were observed at cell borders. The particles in the cell field were associated with larger granules which contained banks of enzymes (mitochondria). Intercellular particles included (1) dense forms 30 millimicra in diameter, identified as polyoma virus; (2) double-membraned forms of 80 or more millimicra containing an internal "nucleoid" and tentatively identified as a leukemia virus; (3) single membraned forms of 60 to 70 millimicra containing an eccentric nucleoid like that in the mammary tumor agent (although smaller than the latter); and (4) single-membraned forms of 65 to70 millimicra containing unidentified particles and vesicles. [13]

The induction of multiple tumors in mice, hamsters and rats by the polyoma virus takes place in a very high percentage of animals when they are inoculated at birth or at a very early age. The relationship of susceptibility to the period just following birth suggests that the absence of immune mechanisms at this time is necessary for tumor induction by the virus. Drs. Vittario Defendi and Hilary Koprowski of the Wistar Institute of Anatomy and Biology have found that the collateral transplantation of adult lymphoid tissue may be an important factor in determining the immunological response of virus-induced tumors. One to 4 day-old hamsters were inoculated subcutaneously with polyoma virus-containing culture fluid. At the same time lymph nodes, spleens and thymuses of 2 to 3 month old hamsters of the same strain were finely ground and suspended in solution, and then injected into the baby hamsters before, simultaneously with, or soon after the latter received the polyoma virus. Eighty-five to 100 percent of the young receiving only the polyoma virus developed tumors, and this result did not appear to be influenced by the introduction of adult tissue at the time the polyoma virus was injected, or afterward. The introduction of polyoma virus after transmission of adult cells very significantly reduced the production of tumors. The specific reasons for this phenomenon are under investigation. [14]

In England, Dr. J. F. A. P. Miller of the Chester Beatty Research

Institute has investigated the viral induction of mouse leukemia by cell-free filtrates injected into newborn and adult mice of several strains. The cell-free extracts were prepared from tissues of AK mice with spontaneous leukemia, and from C3Hf/Gs mice with leukemias induced by the leukemic filtrate, Passage A. The AK leukemic filtrates inoculated into newborn, low-leukemic strain mice resulted in the production of leukemia in 11.8% of 68 C3Hf/PW mice at 7 to 14 months and in 7% of 71 CBA/H mice at 6 to 15 months. No leukemias occurred when these filtrates were inoculated into 34 adult C3Hf/PW mice or 18 adult C3Hf/Gs mice. Passage A filtrate resulted in 26.7% leukemias at 7 to 10 months when inoculated into 45 newborn C3Hf/PW mice and in 100% production of leukemia at 2 to 4 months following inoculation into 87 one to 14 day-old C3Hf/Gs mice. The same filtrate produced 60% leukemias when inoculated into 20 one to 2 month-old C3Hf/Gs mice. Acceleration of the onset of leukemia to 3 to 6 months occurred in 68% of 75 AK mice inoculated at birth with AK leukemic filtrates but in none of 18 AK mice inoculated at 1 to 2 months of age. No leukemia developed in 79 C3Hf/PW mice which received injections by vein at birth of normal spleen and thymus cells from healthy young adult AK mice. [15]

When a mouse receiving a tumor from a mouse of another strain is pre-injected with killed tumor tissue, or with non-living preparations of certain normal tissues from the strain in which the tumor originally developed, the growth of the living tumor transplant is often markedly enhanced. This surprising effect is contrary to anticipated result. It has been variously called the XYZ effect, the enhancing effect, and immunological enhancement. Dr. G. D. Snell of the Jackson Memorial Laboratory and his colleagues have proposed that antiserum produced by the host (due to preinjection with dead cells) blocks certain immune reactions at the cellular level. When antiserum was given together with an antigen consisting of foreign strain lymphoid cells, almost no additional antibody was manufactured by the host animal. This was in contrast to results in control mice receiving only foreign cells, which produced red cell antibody in large amounts. In a confirmatory test, presumptively immune lymph node cells were mixed with tumor cells and injected into tumor-bearing mice, and this inhibited tumor growth. Antiserum given at the same time greatly depressed the immunity of the cells expressed from the draining nodes. [16]

Dr. Armin C. Braun of the Rockefeller Institute has shown that growth of the crown-gall tumor of plants does not necessarily represent an irreversible developmental change in the plant tissue. Single cells of a tobacco plant embryonic tumor were isolated and some were successfully grown in tissue culture. Six of these yielded clones in compact colony form which organized abnormal structures when the clones attained diameters of 0.7 to 1.0 centimeters. Upon the third transplantation of these tissues, atypical leaves and buds developed from several of the tissues of one clone, and this organization persisted upon further retransplantation. Dr. Braun then forced these abnormal tumor buds into the healthy growing tips of tobacco plants. In successful instances the tumor grafts developed into overgrowths of considerable size, and upon continued regrafting, the tumorous tissue grew more rapidly and became more normal in appearance. Flowers were obtained which set seed and the seed gave rise to independent plants which

appeared normal. Dr. Braun considers that somatic mutation is an unlikely explanation for the cause of crown gall.[17]

Cancer morphology and physiology

Deoxyribonucleic acid (DNA) has been prepared by Dr. Giampiero di Mayorca and his associates of the Sloan-Kettering Institute for Cancer Research and the Sloan-Kettering Division of Cornell Medical College, from normal human spleen, from white blood cells (leukocytes) of normal human donors and from patients with chronic lymphatic leukemia and granulocytic leukemia. The various samples of DNA obtained were found to be essentially indistinguishable in base, nitrogen, phosphorus, sodium and water content. However, the samples differed in their chromatographic absorption patterns, and a profound difference was found in the chromatographic profiles of DNA from untreated cancer patients and patients who had been given the cancer inhibiting compound Myleran. [18]

Electron-microscope studies of the breast tissue of mice of the C3H strain have disclosed the appearance of virus-like particles, seen in mice both with the mammary tumor agent and in those presumed to be free of the agent. Drs. Dorothy R. Pitelka, Kenneth B. DeOme and Howard A. Bern of the University of California observe that the presence of these particles in large amounts in agent-free mice was surprising, a circumstance emphasized by the ordinarily low incidence of breast tumors in these laboratory animals. These researchers could find no direct evidence that the particles are related to tumor production, although it was noted that there is a consistent association of the particles with pre-cancerous breast tissue enlargement. [19]

In the attempt to gain a better understanding of human leukemia, it was of interest to Dr. P. C. Nowell of the University of Pennsylvania School of Medicine to know whether the undifferentiated or immature cancer cell types which appear in the circulation are capable of differentiating into mature forms, or if their cancerous state makes them incapable of such maturation. Leukocytes were obtained from the peripheral blood of 3 patients with subacute granulocytic leukemia, 2 patients with monocytic leukemia, and 3 healthy individuals. They were grown in short term tissue cultures. Within a few days, both normal and leukemic cultures consisted of a uniform population of large, undifferentiated cells which divided actively. Periodic agitation or additions of fresh plasma maintained the cells in this undifferentiated state for several weeks. In cultures left undisturbed, development to maturity of the undifferentiated cells occurred within 7 to 10 days after the last agitation or addition of plasma. Immature granulocytic leukocytes differentiated into a polymorphonuclear cell type and leukemic monocytes developed into macrophages and also large giant forms with many nuclei. [20]

The human cancer cell strains, HeLa and L have been experimentally frozen for long periods of time, and their ability to recover and live has been evaluated by Dr. W. F. Scherer of the University of Minnesota. He froze these strains for 4 to 5 years, and studied the effects of freezing velocity on long-term survival. Both HeLa and L cells were recovered and

propagated after storage on dry ice for this period. However, only the HeLa cells frozen slowly over 1 to 1½ hours were viable, an observation which agrees with earlier observations on L cells; this clearly indicates that slow-freezing is superior to quick freezing for long-term preservation of these strains. [21]

Host-tumor relations

Research on the conditions existing between the host and its tumor has been concerned largely with the immunological responses of the host. In the case of transplanted tumors, the antigenic behavior of the latter as a genetically characterized "foreign body" may require the creation of specially defined physiological conditions in the host to influence the antibody (and hence the antiserum) defense responses of the latter.

Dr. Andrew A. Kandutsch of the Jackson Memorial Laboratory has studied the distribution of tumor graft-enhancing antigens in cells and methods for their possible selective enhancement. Ordinarily tumors will grow only when transplanted in an animal of the same strain. He took cells from the mouse tumor Sarcoma I growing in strain A mice and transplanted them in mice of strain B-10 D2. Different portions of the cells were tested for the presece of antigens, and antigenic activity was found to be widely distributed in the subcellular particulate fraction of the cell (nucleus, mitochondria, microsomes and fluffy layer). Dr. Kandutsch also developed a procedure for extracting antigens from these fractions. [22]

Properdin is a protein of the blood serum believed to be a factor in natural immunity against germ and viral diseases, and perhaps also against cancer. It combines with zymosan, an insoluble cell-wall residue from yeast, a fact useful in assay determinations. A comparison has been made of zymosan and bacteriophage assays in serum properdin determinations in normal subjects and in patients with leukemia and lymphoma by Dr. A. L. Baltch and his colleagues of the State University of New York Medical College. The concentrations of serum properdin were studied in 100 normal subjects, 64 patients with leukemia and 80 patients with lymphoma. The average properdin concentrations in normal individuals was 4 micrograms per milliliter, using the zymosan technic and 9 micrograms per milliliter using the phage take-up technique. As determined by the zymosan assay the mean properdin values were low (less than 2 micrograms) in chronic lymphocytic leukemia, myelogenous leukemia and in Hodgkin's disease. As determined by the phage technic the mean properdin titer was also low (less than 2 micrograms per milliliter) in chronic lymphocytic leukemia and active Hodgkin's disease; normal or high titers were found in chronic myelogenous leukemia and inactive Hodgkin's disease. Zymosan and phage assays showed poor quantitative agreement under like conditions, and it may be due to the possibility that properdin is not a single substance. [23]

In a substantial proportion of rabbits bearing Shope papillomas, there is a progressive development from papilloma to invasive and even metastatic cancer. These can be transplanted serially to other rabbits, and it has been found that for many transplant generations papilloma grafts had the

power to immunize the recipient animal against the Shope papilloma virus. Dr. Robert C. Mellors of the Hospital for Special Surgery (New York observes that this finding, together with the recoverability of Shope papilloma virus in cell-free extracts of such cancers under special conditions, indicated that the virus was present in the papilloma-derived tumors, at least for a while. In one line of transplantable cancer, the Vx2 carcinoma, the power to immunize against the papilloma virus was lost after more than eight years of propagation (46 generations) while in another, the Vx7 carcinoma, the power to immunize was still retained after more than 46 generations.[24]

Homozygous lymphomas of mice grow only in animals with the same heredity-factor make-up, while the first succeeding generation (F_1) hybrid gave rise to progressively growing variant sublines, selectively compatible with one or the other of the parental strains. Dr. K. E. Hellström of the Karolinska Institutet Medical School (Sweden) reports that serologic tests showed variants had lost their sensitiveness to cell-destroying antibodies directed against antigenic factors derived from the opposite parental strain. This sensitivity could not be restored by serial passage of the variant subline in the first generation hybrid of origin or in the opposite parental strain for 12 to 17 transfer generation. Homozygous lymphomas and the variants derived from heterozygous lymphomas were slightly more sensitive to the cell-toxic effect of antibodies than were the first generation hybrid lymphomas. It was not possible to select any homologous antibody resistant cells in a tissue culture of a first generation hybrid lymphoma by four consecutive treatments with antibodies directed against one of the parental strain components. The permanence of the changes observed suggests that the mechanism of variant formation is genetic.[25]

Drs. Robert M. Dougherty, J. A. Stewart and Herbert R. Morgan of the University of Rochester School of Medicine and Dentistry have investigated the development of virus-neutralizing antibody in chickens following infection with various doses of the cancer-causing Rous sarcoma virus. High doses of virus caused an initial elevation in host antibody in about 5 days after the development of a tumor weighing about 1 gram. With low doses of virus the time of development of the immune response was less regular, but the antibody present in the blood stream appeared to exert no manifest influence on tumor growth. Passive immunization of chickens with very large doses of the Rous sarcoma virus immune serum produced a transient delay in tumor response when high or low challenge doses of the Rous sarcoma virus were used, and a small reduction of tumor incidence when low challenge doses were used. They conclude that in this case an immune "response" has little if any effect on the growth of Rous sarcoma virus in chickens once the tumor is produced.[26]

A cancer of the kidney (renal adenocarcinoma) in ordinary adult Vermont frogs (Rana pipiens) has been implanted by Dr. Merle Mizell of Tulane University into the tail fin of tadpoles of the same species. After allowing sufficient time for the implant to "take" and grow, the tail was amputated through the tumor. During the regeneration the normal tissues at the cut surface undergo dedifferentiation, and therefore the tumor

portion in this region may be regarded as under the influence of dedifferentiating forces. The tumor grew out into the area adjoining the region of regeneration, but no outgrowths were ever seen invading the region of regeneration. The outermost portion of the tumor tissue in the area of dedifferentiation broke down, although the cells of the tumor implant nearer the body were unaffected. [27]

Epidemiological studies

Certain factors involved in the causation of bladder tumors have been discussed by Dr. Johannes Clemmesen and his associates of the Danish Cancer Registry. Tobacco smoking is considered to be a possible causative factor. Dr. Clemmesen's statistics confirm this impression, and are in good agreement with a retrospective analysis of clinical records from the Roswell Park Memorial Institute. Besides confirming these more preliminary statements, Dr. Clemmensen's investigation has given new information about the association between the smoking of cigarillos and cigars, and bladder tumors. This association seems less pronounced than the association between cigarette smoking and lung cancer, but the frequency of occurrences and the frequency of malignant changes suggest that patients with bladder papillomas should stop smoking. [28]

Drs. John Higginson and A. G. Oettle of the South African Institute for Medical Research in Johannesburg, South Africa have made a survey of cancer in two negro races, the Bantu and Cape Colored. In the Bantu, cancer of all sites is about half as common as in the United States; except for cancers of the esophagus and liver which are significantly more frequent than in the population of the United States and Denmark, whereas tumors of the other parts of the gastrointestinal tract are much rarer. It was found that cancers of the paranasal sinuses are common and may be related to the use of snuff. Frequency studies show further that cancers of the neck of the womb (cervix) were common, but cancer of the uterus itself was almost unknown; breast cancer is much less frequent than in the United States, as are tumors of the bones and soft tissues, lymphomas and leukemias. It was found also that the pattern of cancer in the Cape Colored population is similar to that in the United States negro, and thus differs considerably from that seen in the Bantu. [29]

Diagnosis

The precipitation reaction patterns occuring in agar gel diffusions (Ouchterlony method) have been used by Drs. Elizabeth E. Miller and Peter Bernfeld of the Bio-Research Laboratories, Inc., to demonstrate that anomalous protein was present in the plasma of tumor-bearing mice which is absent in normal control animals. Differences in the concentration of certain proteins, in particular of alpha-globulin, between the plasma of normal and of tumor bearing mice were also evident. In 34 out of 37 instances, tumor-bearing animals were correctly identified by the observation of an extra precipitin line, whereas the remaining observations were questionable for diagnostic purposes. Immunological reactions in another experiment-produced 74% correct analyses of the agar-gel precipitin configurations. The procedure is regarded as a potentially useful

diagnostic tool. [30]

A study of the selective rate of uptake of radioactively prepared formate in cancerous breast tissue growing in tissue culture has been reported by Dr. Sydney Kofman and his associates at the Presbyterian-St. Luke's Hospital (Chicago) and the University of Chicago. This compound was selected because the pathways of its incorporation in proteins and nucleic acids are fairly well known. In the last 3 years, 91 biopsies of tumor tissue from 32 patients with breast cancer have been obtained for evaluation using formate. Dr. Kofman found that the uptake of radioactive formate in normal breast tissue was relatively constant among individuals and in repeated biopsies from the same individual. There was, however, considerable variation in the rates of uptake in tumor tissue. There appears to be some degree of correlation between the rates of uptake of formate in tumor proteins and tumor progression or regression. [31]

Immunotherapy

The induction of tumor immunity with a new filterable agent has been discussed by Dr. Jerome H. Sacks, Richard F. Clark and Richard H. Egdahl of the Medical College of Virginia. The immunizing material, discovered in their laboratories, was capable of reproducing itself, and was responsible for causing acute infectious hemolytic anemia. It was tested on pen-bred, young adult virgin female Sprague-Dawley and Holtzman rats which were appropriately injected with the agent, now serving as an antigen, thus immunizing the rats. The agent-free tumors used were the Walker 256 carcinoma, the Novikoff hepatoma, the Murphy-Sturm lymphosarcoma and a spontaneous fibrosarcoma which originated in the laboratories of these researchers. These tumors were injected into the abdominal cavity of immune and non-immune (control) test animals. In one experiment (9 animals) there was 22% complete protection from the transplantation of the Novikoff hepatoma in the agent-immune animals. A combination of the results of this and another test demonstrated that 13 of the 24 agent-immune, tumor-inoculated animals were totally protected from transplantation of the tumor (there were 100% "takes" in non-immunized control animals). These differences are held to be statistically significant. In 15 immunized animals given the Murphy-Sturm lymphosarcoma, 13 animals living are tumor free. Immunized animals inoculated with the Walker 256 carcinoma responded with 4 protected animals out of 9. In the other 5, four of the tumors were of an unusual appearance and of limited growth. Six of 10 animals injected with the fibrosarcoma are tumor-free. These researchers conclude that induced immunity in the experimental animal has been found effective aginst every tumor tested; and is specific for tumors and non-injurious to normal cells. [32]

Leukemia in adult Swiss strain mice has been linked to a filterable agent resembling a virus, by Dr. Charlotte Friend of the Sloan-Kettering Institute for Cancer Research. As in the case of other virus-caused diseases, the best means of controlling it is to produce a vaccine specific to the disease, and Dr. Friend has succeeded in producing an antiserum capable of neutralizing the agent. The greatly enlarged spleens of leukemic mice were homogenized and a suspension of the dissociated cells was made. In some instance the supernatant fluid above the resultant sedimented cellular mass

was used as an antigen without further modification and in other instances it was filtered, and was used to immunize both rabbits and Swiss mice. The blood antiserum of these experimental animals was withdrawn after appropriate immunization and was combined with formalin to provide a vaccine. The vaccine induced a significant degree of immunity against the agent, since approximately 80% of the vaccinated mice were immune when exposed to the agent.[33]

Radiotherapy

The effects of x-irradiation upon rat ascites tumors have been explored by Dr. Yahiro Irako of the Medical Institute of Sasaki Foundation (Japan). Twenty different strains of the rat ascites hepatoma were studied, in an attempt to find individual differences in radiosensitivity existing among these tumors which were ultimately derived from one normal ancestral liver cell. With constant dosage, the results showed that (1) even tumors derived from one and the same ancestry were unlike in their radiation sensitivity; (2) the radiation sensitivity of tumors is related to their growth velocity, that is, the slow-growing tumors are more radiosensitive than the faster growing ones; (3) x-irradiation induced more remarkable damages in the tumors showing more marked variation in cell appearance than in tumors consisting of uniform cells; and (4) no correlation was found between the radiosensitivity of tumors and their response to the cancer inhibiting agent nitrogen mustard. [34]

In the past 20 years it has become common custom to treat endometrial carcinoma in one of two ways; (1) radiation, and (2) the application of radium with subsequent surgical removal of the entire reproductive tract including the vaginal apex. Drs. Langdon Parsons and Frank Cesare of the Massachusetts Memorial Hospital and The Boston University School of Medicine have made a clinical analysis of 62 cases of this type of cancer treated by the extensive surgery referred to (the Wertheim hysterectomy). Bilateral dissection of the white-cell lymph nodes of the pelvis was carried out in 50 of these 62 patients while in the remaining twelve no pelvic lymph node dissection was done. Fifty-two tumors were primary, while in 10 other cases previous irradiation had been received. Muscular tissue of the womb had been invaded by the cancer in 37 cases or 60% of the total group. Among the 50 patients undergoing node dissection, 4 had lymph node involvement, an incidence of 8%; and in 37 patients wherein the musculature was cancerous, 4 or 11%, also had cancer in the nodes. Of the latter, 2 are alive and well at 2 years. Eighty-one percent of the operated patients survived 5 years; and those with metastases to the nodes had a 5 year survival rate of 71%.[35] In the treatment of cervical cancer these researchers and Dr. Gilbert Friedell stress that more emphasis on adequate treatment of the disease at the primary site is indispensable for effective results. [36]

The protective effect of bone marrow cell infusions subsequent to total body x-irradiation to destroy leukemia has been investigated by Drs. Isaac Djerassi, Robert Woodruff and Sidney Farber of the Children's Cancer Research Foundation. It was found that the survival of mice exposed to lethal total-body x-ray doses can be improved considerably by single or multiple intravenous administrations of bone marrow cell suspensions

derived from mice of the same inbred strain. Large quantities (8 to 10 million) of nucleated bone marrow cells were used for each infusion. The high percentage of surviving animals protected with one single dose of bone marrow given after 4 to 5 days appears to indicate that close to optimal protection can be achieved at a relatively late period after exposure to x-irradiation by the infusion of large amounts of bone marrow cells. [37]

In another research project undertaken at the Children's Cancer Research Foundation, Drs. Giulio J. D'Angio, Sidney Farber and Charlotte L. Maddock have tested the potentiation of x-ray effects by the antibiotic actinomycin D. The agent was given intravenously or intraperitonially to mice of the CAF strain, in single doses of 150 micrograms per kilogram of body weight or in proportional amounts over 3 days. The x-ray doses were 1,000 roentgens (air) in one exposure or 1,200 roentgens (air) in 3 days (400 roentgens daily). Enhanced responses were obtained in normal tissues and in certain cancers when the antibiotic was injected by vein. Smaller doses of x-rays were possible and healing proceeded more rapidly. Certain notoriously resistant cancers such as melonoma have shown a limited favorable response to this combined therapy. [38]

The physical aspects of a 2,000,000 volt x-ray therapy unit in the treatment of cancer have been described recently by Drs. Kenneth A. Wright, Basil S. Proimos and John G. Trump of the Lahey Clinic and the Massachusetts Institute of Technology. This high voltage radiation penetrates a deep tumor more effectively and scatters less radiant energy out of the directed beam into the surrounding normal tissue. At the Massachussetts Institute of Technology the 2 mev Van de Graff electrostatic accelerator delivers 80 roentgens per minute at a distance of 100 centimeters. An important clinical advantage of this particle accelerator arises from the fact that the area of impact of the high energy electrons on the target is usually less than 3 mm. in diameter. Small tumors of the head and neck, such as pituitary cancer and intrinsic cancer of the larynx thus present almost ideal situations for rotational therapy with this type of radiation. [39]

Hormonal therapy

The pituitary gland has been removed in operations upon 53 carefully studied advanced breast cancer patients under the care of Drs. A. G. Jessiman, D. D. Matson and F. D. Moore of the Harvard Medical School and the Peter Bent Brigham Hospital. They report that in 47 of these patients the course of the disease was favorably altered. The survival time of those manifesting an objective response was five times as long as that of those whose disease was not altered by the operation and the longest remission obtained was for 15 months. The investigators believe that the most favorable type of patient for hypophysectomy appears to be a woman who is less than ten years post-menopausal, whose cancer has spread to the skin, lymph nodes, skeleton or lung, but not to the brain, liver or the viscera generally. Also she should have good heart, kidney and lung function and a demonstrably hormone-sensitive tumor. [40]

Patients with chronic lymphatic leukemia and malignant lymphoma characteristically respond very well to radiation therapy for a time, but most of them develop anemias as they enter the terminal stage. Dr. John G. Freymann and his associates at Harvard University are of the opinion that long-term, continous therapy with adrenal corticosteroids constitutes an effective palliative procedure after these patients become unresponsive to x-ray therapy. A minimum of 40 milligrams of prednisone or 200 milligrams of cortisone daily were given to each patient over a period of not less than 3 months. These investigators have treated 19 such patients with this therapeutic regimen and obtained beneficial responses in 15. Corticosteroids exert their effect by directly stimulating the bone marrow to greater red cell production, and this therapy must be maintained for the life of the patient.[41]

A new compound, delta'-testololactone, has been tested for its effectiveness in treating cancer of the human breast by Drs. A. Segaloff, J. B. Weeth, E. L. Rongone, P. J. Murison and C. Y. Bowers of the Alton Ochsner Medical Foundation, Tulane University and the Louisiana State University, working under the protocol of the Cooperative Breast Group of the Cancer Chemotherapy National Service Center. This agent, although similar in molecular structure to male sex hormone, lacks hormonal properties. Twenty-three patients with advancing breast cancer were given delta'-testololactone in doses of 100 milligrams intramuscularly 3 times weekly. Seven of these patients showed objective regression of metastatic cancer, without evidence of virilization and without creating hormonal imbalances in the treated patients.[42]

Chemotherapy: Clinical Research

The potentiating effect of the vitamin metabolite citrovorum factor upon the effectiveness of the antitumor agent methotrexate has been tested by Dr. Robert D. Sullivan of the Veterans Administration hospital, New York City, and Drs. Edward Miller and Marguerite P. Sikes of the Sloan-Kettering Institute for Cancer Research. The methotrexate was administered through a catheter placed in an artery ending near the tumor, while the citrovorum factor was injected intramuscularly. Eighteen patients received large amounts of methotrexate and partial or complete regression of the cancer was noted in 10 patients. The intermittent use of citrovorum factor permitted the body to tolerate larger amounts of methotrexate than otherwise would be the case.[43]

Drs. Rudolph Mrazek, Steven Economou, Gerald O. McDonald, Danely P. Slaughter and Warren H. Cole of the University of Illinois College of Medicine have reported on the results of prophylactic and adjuvant cancer chemotherapy with nitrogen mustard associated with surgery in the treatment of cancers of the breast, large intestine and rectum. Nitrogen mustard is administered with the hope of destroying cancer cells liberated into the blood stream at the time of operation or shortly before, and that are beyond the scope of surgical resection. This combined treatment was given to a group of 136 patients with cancers of the breast, colon and rectum. One-half of these patients received nitrogen mustard immediately after surgery; the other half served as controls. This treatment resulted in bone-marrow depression

and associated reduction in white blood cells in varying degree in the majority of patients, particularly those who were fat. The white blood cell reduction was compensated for by transfusions, and no post operative deaths could be attributed directly to the nitrogen mustard therapy. In the patients with breast cancer, only 5 recurrences and 1 death from cancer have occurred in the treated breast cancer patients, compared with 12 recurrences and 7 deaths in the controls. At the date of the report statistical results in the colon and rectal group were too few to compare.[44]

Chlorambucil is an anticancer agent related to nitrogen mustard, and its clinical effectiveness has been investigated by Drs. Daniel G. Miller, Henry D. Diamond and Floyd F. Craver of the Sloan-Kettering Institute for Cancer Research. They tested it against Hodgkin's disease, chronic lymphocytic leukemia, lymphosarcoma, chronic myelocytic leukemia and reticulum cell sarcoma. In contrast to usual treatment patters, each patient was given maximal doses of the drug until either a marked response was obtained or side effects precluded further dosage. A marked favorable response to treatment was manifest by regression of all evidence of the disease in a significant number of patients under conditions of the experiment. Of 32 patients with Hodgkin's disease 11 or 34.4% had a marked response; in 6 patients with lymphosarcoma, all responded to this degree; in 19 patients with chronic lymphocytic leukemia 6 gave a like response; 5 of 6 patients with chronic myelocytic leukemia benefited likewise and 2 of 5 patients with reticulum cell sarcoma manifested this degree of improvement. It is concluded that chlorambucil is an effective anti-cancer agent for treatment of these specific tumors.[45]

The clinical effectiveness of chlorambucil as compared with that of myleran in the treatment of human cancer has been evaluated by the Southwestern Cancer Chemotherapy Cooperative Study Group. Studies were made in 40 patients with chronic lymphocytic leukemia and in 42 patients with chronic granulocytic leukemia. In the lymphocytic leukemia chlorambucil produced good or excellent therapeutic responses in 15 of 23 patients. None of 17 patients treated with myleran responded this well. In the cases of granulocytic leukemia, myleran produced good or excellent therapeutic responses in all 21 patients treated. Only 12 of 21 patients given chlorambucil had comparable responses.[46]

The expanding development of new chemotherapeutic agents for use in the treatment of acute leukemia has created a need for both extensive clinical trials of the antileukemic drugs and for the establishment of methods of evaluation of subsequent clinical responses. Drs. R. M. Heyn, C. A. Brubaker, J. H. Burchenal, H. G. Cramblett and J. A. Wolff of the Leukemia Chemotherapy Cooperative Study Group A, representing investigators from a number of institutions throughout the United States, have reported upon a comparison of the effectiveness of 6-mercaptopurine with that of a combination of 6-mercaptopurine and azaserine in the treatment of acute leukemia in children. The cases were accumulated in a period of 15 months. In 125 cases of previously untreated acute leukemia in children, no significant difference was seen in the percentage of complete remissions obtained when 6-mercaptopurine was used alone, or when 6-mercaptopurine and azaserine were used in combination. The duration of the remissions obtained

with the combined therapy was not significantly longer than when 6-mercaptopurine was used alone.[47]

An important limitation to the use of nitrogen mustard as an anticancer agent has been its toxicity for normal tissues of the stomach and intestinal tract and the blood-forming system. Dr. W. Gerald Austin of Harvard University and his associates have used a perfusion technique which permitted greater amounts of the drug to be administered to patients with lower abdominal tumors than otherwise would be the case. The aorta and vena cava were clamped and both legs bound by pneumatic cuffs inflated around the upper thighs. A tube was inserted into the large artery of the upper leg above the cuff and one was inserted also into the vein of the same region. The tubes were attached to a system which circulated and oxygeneted the blood in this closed circuit. Under these conditions high doses (1.0 milligrams per kilogram of body weight) were given safely. Four patients out of 7 treated in this manner showed cancer regression, with encouraging results.[48] Dr. Anthony P. Monaco, working with Dr. Austin, tried similar therapy upon 2 patients with a bleeding type of cancer, Kaposi's sarcoma, which at first is most often found on the hands and feet. Both persons showed prompt regression of their lesions, without toxic side effects.[49]

Further experimental development and clinical application of regional perfusion techniques of isolated body areas with chemotherapeutic agents in the treatment of cancer have been undertaken by Dr. Edward T. rementz of the Tulane University College of Medicine and his colleagues. They have treated 40 patients with cancer using an extracorporeal circuit with a closed circulatory area involving the tumor. Eight patients had malignant melanoma, 14 had sarcoma and 7 had carcinomas of the rectum, uterus or bladder. Perfusion of the limbs, pelvis and lung with chemotherapeutic agents could be performed safely for periods up to 1 hour. The results indicate that the methods employed are useful for palliation of regionally confined lesions, but not for advanced lesions. The most encouraging results were obtained in the treatment of malignant melanoma with perfusion of phenylalanine mustards.[50]

Chemotherapy: Experimental Animal Research

An anti-cancer agent termed calvacin has been isolated from extracts of a giant puffball mushroom (Calvatia gigatea). Drs. H. Christine Reilly, K. Sugiura and C. Chester Stock of the Sloan-Kettering Institute for Cancer Research, working in collaboration with Dr. J. F. Roland and his colleagues of Armour and Co. have developed a submerged fermentation process as a more convenient and reproducible source of calvacin. The researchers at the Sloan-Kettering Institute determined the antitumor activity of test samples in the standard Sarcoma 180 mouse assay. Calvacin was found to possess antitumor activity against 14 of 24 various mouse, rat and hamster tumors, notably against the free-cell or ascitic type of Sarcoma 180, Sarcoma MA 387 and the Crabb hamster sarcoma. Present knowledge of the chemical nature of calvacin indicates that it is nondiffusible, basic mucoprotein.[51]

In a program of continuing studies on the physiological response of tissues to chemotherapeutic doses of anticancer agents, Dr. Charles

Heidelberger of the University of Wisconsin Medical School and his associates have investigated tissue response to certain of these. Experimentation was carried out in tissue culture with the free-cell Ehrlich ascites cancer, which can be resistant in some cases and sensitive in others, to a class of chemical compounds called fluorinated pyrimidines. These agents may interfere successfully with cell chemical reactions in sensitive tumor cultures, in particular the take-up of formate or thymidine for the production of an important cell compound, thymine; and likewise the incorporation of uracil or of orotic acid into ribonucleic acid. These reactions can be followed by using formate made radioactive by incorporation into the molecule of carbon-14. Although in resistant cells there is a decreased conversion of the pyrimidine 5-fluorouracil into 5-fluorouridylic acid and incorporation into ribonucleic acid, the amount of 5-fluoro-2'-deoxyuridine-5'monophosphate produced was equivalent in resistant and sensitive cell types. An enzyme, thymidylate synthetase, was found to be involved; and in sensitive cells only, the enzyme's activity was inhibited by the fluorinated pyrimidine. Why this enzyme is affected in some cells but not in others, remains to be determined.[52]

Nitrogen mustard has achieved wide use as a palliative agent in the treatment of a few forms of far-advanced cancer. Because of its toxicity in high doses, Dr. Laurens P. White of the Stanford University School of Medicine has investigated conditions under which stronger, and hence more cancer-inhibitory doses of nitrogen mustard could be given without undue toxicity. He has found that norepinephrine (which constricts arteries and arterioles of the spleen) when given to experimental animals before the administration of nitrogen mustard, produces a high degree of protection from depression of bone marrow cellular activity which usually follows dosage with nitrogen mustard. Dogs and rats whose spleen had been removed were much more resistant to the toxic effects of this anti-cancer agent than were controls, with less bone marrow depression and fewer deaths. Extirpation of the spleen plus norepinephrine gave the highest degree of protection.[53]

A biological assay system for testing cancer-inhibiting compounds has been developed by Dr. Veronica Armaghan of the University of Miami when it was observed that the reduction in effectiveness of compounds tested at frequent intervals in the course of development of IRC 741 leukemia in Fischer rats was not a uniform and gradual decline. The compounds tested were nitromin, Elderfield mustard, tetramin, triethylene thiophosphoramide, mannitol mustard, nitrogen mustard, amethopterin, urethan, 2,5-bis (1-aziridinyl)-3,6-dipropoxy-p-benzoquinone (E-39) and 2,5-bis(1-aziridinyl)-3,6-bis(2 methoxyethoxy)-p-benzoquinone (A-139). By initiating treatment at intervals of 1, 2, 4, 6, 8 and 10 days after transplantation it was possible to show differences in response to compounds which are not discernible when testing is limited only to early and established tumors. Treatment initiated on the first day with nitromin, Elderfield mustard and tetramin resulted in 80% cures; when treatment was started on the sixth day, only tetramin effected a partly favorable response (10% cures). Similar results were obtained with the other agents, but the pattern in each case was highly characteristic of the agent.[54]

Drs. F. Berge, J. M. Johnson and J. A. Stock of the Institute of Cancer Research, London, have synthesized the compound beta-[para-di-(2-chloroethyl) aminophenyl] -DL-beta-alanine and report that it has anticancer properties. Preliminary experiments showed that the Walker rat carcinoma 256, an experimental tumor, was completely inhibited by 20 milligrams of the drug per kilogram of body weight. It was likewise quite effective against Sarcoma 180. The agent was injected peritoneally.[55]

A new anticancer agent, cyclophosphamide, has been tested by Drs. E. T. Kovacs, S. Ohno and R. Kinosita of the City of Hope Medical Center, for its effects on mouse leukemia. Cyclophosphamide was injected into a leg muscle of mice carrying transplanted tumor cells. The compound acted quickly against L4946 leukemia. Microscopic examination of tissues showed practically no viable tumor cells remaining when treatment was started 4 days after transplantation, although fatal recurrence ensued eventually. With C 1498 leukemia, response to treatment was similar, except that the pallative period was not so long.[56]

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Summary of Research Grant Transactions for Fiscal Year 1960

FIELDS	NUMBER	AMOUNT
Cancer causation	288	$ 5,294,330
Tumor growth	227	4,066,891
Host-tumor relationship	77	1,454,980
Epidemiology	42	631,498
Diagnosis of cancer	81	1,358,909
Therapy of cancer:		
Programmed grants in chemotherapy	166	2,845,593
All other	461	11,034,454
Basic research and all other including grants covering multiple fields	456	7,238,135
Clinical research centers (therapeutic and metabolic)	-	500,000
Scientific evaluation and planning grants	5	280,000
Reimbursement to "National Institutes of Health Management fund"	-	90,000
Total Grants	1,803	$34,794,790

V. The Postdoctoral and Special Fellowships Awards Program

The Fellowship program of the National Cancer Institute supports the training of properly qualified candidates for research and academic positions in basic science and clinical fields pertinent to cancer so that an increasing number of qualified investigators will be enlisted in the attack on cancer. The program finances post-doctoral and special fellowships, and provides supply grants to help defray the costs of supplies and similar educational expenses.

Postdoctoral and special fellows broaden their research training and experience under the supervision of or in association with an established scientist, with the objective of preparing them to become investigators and teachers in the research and educational institutions of the country. The currently recognized shortage of adequately trained research personnel to staff these institutions emphasizes the need for increasing the pool of research manpower on which progress in scientific research depends. Approximately 90% of the scientists previously trained under this program are now actively engaged in research or teaching or both, of subjects related to cancer.

Research papers published by fellows of the National Cancer Institute have reported studies on the physiological chemistry of the ribonucleic acids and their associated enzyme systems; details of embryogenesis and developmental mechanics; genetic analyses of x-irradiation; tumor induction and dissemination, and related tumor phenomena, among other experimental

results.

The characterstics of soluble enzymes which convert the pyrimidine nucleoside, thymidine, phosphates and deoxyribonucleic acid have been studied by Dr. F. J. Bollum, a postdoctoral Fellow working in the laboratory of Dr. Van R. Potter of the University of Wisconsin. He reports on a technic for comparing deoxyribonucleic acid synthesis in regenerating rat liver in vivo and in cell-free supernatant fractions from the same animal in tissue culture. The enzymatic incorporation of thymidine into deoxyribonucleic acid was found to appear at the same time in vitro (18 hours after operation) as deoxyribonucleic acid synthesis in vivo but continued to increase over the period of 18 to 30 hours, while deoxyribonucleic acid synthesis in vivo was decreasing (24 to 30 hours).[1]

Lewis B. Barnett, a predoctoral Fellow at the State University of Iowa, has determined the isoelectric point (neutrality) of the enzyme ribonclease adsorbed on glass, Dowex 50 cation-exchange resin, Dowex 2 anion-exchange resin, paraffin and Nujol in various buffer systems at an ionic strength of 0.05 and 25°. Comparison of these data with the isoelectric points obtained by the moving boundary (Tiselius) method indicates significant shifts after adsorption. These shifts depended upon the buffer system employed and the adsorbing medium. The pH range from all data was 4.85 to 9.1, depending upon the technic employed, and was characteristic for each medium.[2]

A method has been described by Dr. Abraham M. Stein of Brandeis University, a postdoctoral Fellow working with Drs. Nathan O. Kaplan and Margaret M. Ciotti, for the assay of the enzyme transhydrogenase activity through the use of the acetylpyridine analogues of diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN). The transhydrogenases of rat liver and beef heart promote the reaction between reduced triphosphopyridine nucleotide (TPNH) and the acetylpyridine analogue of DPN at a much faster rate than the analogous reverse reaction, the reaction of reduced diphosphopyridine nucleotide (DPNH) with the acetylpyridine analogue of TPN. Thyroxine and triiodothyronine have been found to inhibit the reaction TPNH and the acetylpyridine analogue of DPN, whereas these compounds do not influence the reaction of DPNH with the acetylpyridine analogue of DPN.[3]

An autoradiographic and isotopic study has been made of the synthesis of deoxyribonucleic acid, ribonucleic acid and some nuclear proteins in the root tip nuclei of the well known plant called spiderwort (Tradescantia). J. E. Sisken, a predoctoral Fellow of the National Cancer Institute at Columbia University reports that during the growth of the nucleus in early interphase, there is no incorporation of tritium-labeled thymidine into deoxyribonucleic acid. After the nucleus reaches a certain size, however, there is incorporation into deoxyribonucleic acid with little or no further increase in nuclear volume. Other tests indicate that the two nucleic acids, deoxyribonucleic acid and ribonucleic acid, are not synthesized together. Ribonucleic acid is synthesized when an increased in nuclear volume occurs, and in general before deoxyribonucleic acid.[4]

The separation and characterization of the diverse, larger molecular components of deoxyribonucleic acid has been undertaken by Dr. Herbert P. Pahl of the Sloan-Kettering Division of Cornell Medical College. The fractionation technique employed involved the use of the anion exchanger ECTEOLA. Deoxyribonucleic acid was quantitatively recovered from human white cells derived from a patient with acute myeloblastic leukemia. This chromatographic procedure has been found to be highly reproducible, and differentiates deoxyribonucleic acid from different sources. It would appear that these various species of deoxyribonucleic acid differ in their intrinsic molecular structural patterns.[5]

The details of implantation of the early rabbit embryo (blastocyst) in the wall of the uterus has been reported by Dr. Bent G. Böving of the Carnegie Institution of Washington. Rabbit blastocyst attachment to the uterus depends on three principal mechanisms: muscle, adhesion, and invasion. Uterine muscle transports the blastocysts from the uterotubal junction to their lodging place and holds them there. The uterine reaction appears to depend on progesterone and probably an absence of estrogen. At implantation, adjacent muscles become disposed in bands (like an incomplete sphincter) that grasp the blastocyst and hold it. The developing embryonic membrance, the trophoblast, then adheras to and invades uterine tissue. Invasion is believed to be associated with high local uterine vascularity and appropriate ionic concentration due to carbon dioxide or other physiologically significant materials.[6]

The growth behavior of the bud tips (apical meristems) in corn has been the subject of a genetic and morphogenetic analysis by Dr. Otto L. Stein, a Research Fellow of the National Cancer Institute at the Montana State University. The purpose of the investigation was to find some relationship between growth, histogenesis, anatomy and genetic damage in irradiated seedlings. Embryos of Yg2/yg2 genetic constitution were irradiated and the number of yellow-green sectors per leaf was scored at maturity. The yellow-green phenotype results from the loss of one end of chromosome 9, and is a direct index for loss of genetic material in vegetative cells. In general, the frequency of sectors increase with dose. The growing apex, however, appears to manifest an all-or-none phenomenon. At the critical radiation dose the entire apex begins to collapse; sublethal doses produce no apparent significant change.[7]

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Summary of Fellowships Awarded in Calendar Year 1960

TYPE	NUMBER	AMOUNT
Full-Time:		
Predoctoral*	108	$ 341,443
Postdoctoral	168	1,051,024
Special	20	174,607
Total, full-time fellowships	296	1,567,074
Part-time medical nursing and public health students	272	176,256
Supply grants and specisl allowances	72	32,285
Total Awards	640	$1,775,615

*Predoctorate and part-time fellowships financed by the National Cancer Institute in 1960 will be transferred to the Division of General Medical Sciences. The National Cancer Institute will use the funds released by the transfer of these programs to expand existing programs and inaugurate the senior and career development programs.

VI. Training Grant Program

The objective of the National Cancer Institute training grant program is to increase the number of well-trained scientists working in clinical biological, chemical and physical disciplines and areas pertinent to cancer research. It is designed to alleviate the shortage of qualified personnel, to increase the research potential and to replace present investigators who are being lost to cancer research through death and retirement.

The National Advisory Cancer Council Committee on Research Training believes that the needs are so widespread in nearly all pertinent fields that none should be excluded as a potential area for the training of scientists who will contribute to new knowledge on cancer and its control. At present special emphasis is being directed towards increasing the number of well-trained, specialized investigators in clinical research on cancer chemotherapy and pathophysiology, biochemistry with emphasis on steroid physiological chemistry, biophysics, pharmacology, genetics, analytical

cytology, virology, immunology, radiobiology and radiotherapy. Training may be wholly within a discipline, interdisciplinary, interdepartmental or interinstitutional.

Graduate training grants allow the director of a training center to select the trainees and to set the trainee stipends in line with the policies of the institution. In addition to stipends, the training grant may provide for additional staff, research equipment and supplies, and other costs essential to the training of research personnel.

This graduate training grant program is complementary to, and does not replace other procedures for training research, academic and clinical investigative personnel under the fellowship program previously described. It offers a means of expanding facilities and staff for scientific program training, and provides for flexibility in meeting variations in the needs of training centers.

The program has created much needed and sought after opportunities for stimulating and attracting advanced students at all states of development to explore new fields, learn new techniques and expand their horizons of knowledge and interest. In many instances, training has been given to individuals who have received no direct financial assistance from another type of grant.

Many of the trainees participate in special courses in steroid biochemistry and endocrinology, and in genetics, histochemistry and other fields. Such courses may extend from a few weeks to 6 months or more. Other trainees participate in research projects as junior collaborators associated with senior investigators. Again, others may engage in more or less independent research on their own problems but under the guidance of a senior investigator.

At the end of June 1960, that part of the Training Grant Program which provides stipends for predoctoral students was transferred to the Research Fellowship Review Branch because of the more general basic research and technological approach predominant in the applications considered. Stipends for postdoctoral and other trainees have been awarded in amounts up to $9,500 a year. In addition to stipends for experimental studies the grants have provided supplies, equipment and facilities to meet the needs of the expanded program.

From cancer investigators who have had experience with these programs, from program directors and research or educational administrators, from trainees and from lay observers, have come unsolicited enthusiastic statements of the value of these training programs in their initial stages. There is a great awareness of the value of scientific training at the present time, and the training program of the Institute is taking advantage of the opportunities provided to stimulate this phase of the over-all cancer research program.

There were 52 active training grants in 1960, and the total funds

awarded were $3,073,238.

VII. Viruses and Cancer Program

During the past year the research grant support relevant to viruses and cancer has grown to over $5.6 million, representing 153 projects devoted either wholly or in some significant part to this area of investigation. Following advice of the Viruses and Cancer Panel, several steps have been taken to insure the security and promote the effectiveness of this investment through provision of research tools and services.

Among the most pressing needs of virologists are the requirements for living reagents of the highest quality for their research. Only living cells support the growth of viruses, yet lines of cultured cells certified as to their characteristics and purity are not yet available.

Supported by research grants, a cooperative program of cell culture studies is now underway to remedy this situation. One of these grants established the Cell Culture Collection Committee early this year. Its first goal is through the work of its member laboratories to characterize mammalian cell lines and set up certified seed stocks free of contaminating microorganisms and viruses so that investigators in cell and virus work may have the cell line as a high grade certified reagent. Preliminary work in the laboratory on candidate cell lines has shown the feasibility of this program.

In order to safeguard seed stocks of these certified cell lines for an indefinite period of time the Committee has endorsed the use of ultra-refrigeration and has stimulated the development of liquid nitrogen refrigerators for laboratory use. These operate at temperatures colder than -170°C, and are independent of electric power.

Current participants in the new cell program include the Committee's administration, two research laboratories, and the American Type Culture Collection in Washington, D. C., as a central, national repository for the certified cells.

Virologists can now isolate viruses rather easily and rapidly in cell cultures and in test animals, but to coordinate and correlate the information attained with that already known, the viruses must be identified, either as ones previously studied or as new agents. The only method having broad utility and dependability is serologic testing, but the reagents for such tests are not certified, extremely expensive, very limited in supply, and are not commercially available. Generally speaking, each laboratory makes its own. No laboratory can contemplate identifying viruses on a routine basis without first expending a very great effort and much money in the production of typing antigens andantisera. Such reagents, produced in individual laboratories, rarely are cross-checked, except as may be done by a few other laboratories in the course of using reference quantities in their own work, or in occasional cooperative studies.

In order to remedy this most severe shortage of tools for virology a large scale program was started to supply certified typing reagents, first for the viruses of man, and then as soon as they can be developed, the viruses of those laboratory animals used in virological work, especially the mouse.

As of July 1, a contract for $380,000 was begun with the Kansas University Endowment Association for the production of such serologic typing reagents as are necessary to identify the enteroviruses of man. This is a group of over sixty viruses including polio. The need for consultant direction of the enterovirus program has led to the establishment of the Enterovirus Committee, which is now actively serving the National Cancer Institute, just as those scientists have served the National Foundation in years past.

The Viruses and Cancer Program also plans to have typing reagents produced for other viruses of man. Part of this task, that of the adenoviruses, has been assumed by the National Institute of Allergy and Infectious Diseases. Other segments may be done by other agencies and by the National Cancer Institute.

Like the cell, the virus, and the viral typing reagent, the laboratory animal is a major part of the virologist's study system. At present virologists must work with animals which are already carrying many viruses, and for which tests cannot be made, yet such extraneous agents may invalidate their research. In order that studies may be done in a clean system, and thereby be valid in their conclusions, specifically "virus-defined" animals must also be made available.

To accomplish an upgrading of the laboratory animal and establish new standards for them and for the methods and facilities needed for their handling, a Committee on Laboratory Animals has been recommended by the Viruses and Cancer Panel and by the National Advisory Cancer Council.

To the investigator trying to link viruses to cancer in man, a most important research material is human tissue.

Under leadership of the Viruses and Cancer Panel, the staff and ad hoc consultants have inquired into the problem of human tissue procurement for virus-cancer research and have found that such research is being held back because these materials are not now readily available. The problems cover the selection, removal, collection, preservation, and distribution of mature and embryonic tissues, both normal and neoplastic. The virologist seeks a tissue sample uncontaminated by the patient's own viruses and microorganisms and consisting of living cells rapidly removed and maintained alive with whatever viruses may be in the sample originally. Such material is not now routinely secured as a part of any surgeon's or pathologist's procedure, and so requires that they be carefully informed and assisted if such a program is to be successful.

To carry forward a bold program along the lines set forth, the National Cancer Institute has begun to staff the Viruses and Cancer Program in response

to the advice of its consultants on the Viruses and Cancer Panel and the National Advisory Cancer Council. Within the past year three positions have been set up as Chiefs, respectively, of the Laboratory Animal Program, the Human Virology Program, and the Cell Culture Program.

At its meeting in November 1960, the National Advisory Cancer Council reviewed the Institute's current achievements under the Viruses and Cancer Program, and endorsed a considerable expansion of it, with full appreciation of the great magnitude involved.

Several very inclusive recommendations were made by the council, covering various areas of research convergent on cancer virology.

During 1961 implementation of the many objectives of the Viruses and Cancer Program will require increasing staff effort and support by both the research grant and the contract mechanism.

Among the newer areas to be emphasized are investigation of the principles and pilot development of facilities required for isolation of experimental animals used in viral research, training of virologists and other investigators, provision for a more rapid and more inclusive exchange of information among research people, promotion of virus-tumor research with primates, development of certain technical equipment, and encouragement of research in viral diagnostic methods.

ACTIVITIES OF THE INTRAMURAL RESEARCH PROGRAM OF THE NATIONAL CANCER INSTITUTE, 1960

INTRODUCTION

The staff of the Institute continued to make important research contributions of a very high order during 1960. The tremendous scope of the activities, their complexity, and the great numbers of important findings make it especially difficult to summarize adequately the work, and much additional knowledge, often at a rather preliminary stage, is not included in the report. Both the collaborative groups and the individual scientists continue to be highly productive, and the most fruitful collaborations are those established by the individual scientists themselves finding a common meeting ground in research problems of joint interest. The attempt to provide a research atmosphere most conducive to sound research programs must take into account the delicate balance between the needs of the Institute as a whole and those of the individual. The ideal balance, particularly in a large organization with its many rules, regulations, clearances and multiple channels of communication, is often difficult, if not impossible, to attain. Nevertheless, the research accomplishments made and a sense that they are often of high significance for society make the considerable effort directed toward this end worthwhile.

At mid year, a number of changes occurred in several key positions in the Institute. Dr. J. R. Heller, who had been Director for 12 years, left to assume the Presidency of the Memorial Sloan-Kettering Cancer Center. Dr. G. Burroughs Mider, who had served as Associate Director for Research for 8 years, joined the Director's Office at NIH as Director of Laboratories and Clinics, NIH. Dr. Kenneth M. Endicott assumed the position of Director, NCI, following two years as Associate Director, NIH. The responsibilities for the clinical and nonclinical intramural programs, which had both been assumed by Dr. Mider, were divided. Dr. C. Gordon Zubrod, who has been the Clinical Director of the NCI since 1954, assumed responsibility for the clinical programs, including the Surgery, Endocrinology, General Medicine and Radiation Branches. Dr. Carl G. Baker, who had been Assistant Director with Dr. Heller for 2-1/2 years, assumed responsibility for the nonclinical aspects of the intramural program, including the Laboratories of Biochemistry, Biology, Physiology, Chemical Pharmacology and Pathology, and the Pathologic Anatomy Branch.

These organizational changes and the impending retirement of Dr. Howard B. Andervont as Chief of the Laboratory of Biology brought about extensive discussions of the direction the Institute should take, of the areas of emphasis and de-emphasis, and of intraorganizational relationships. One change is the expectation that scientists on the intramural staff will participate more actively in some of those programs, formerly classified as "extramural" or in the "gray area," for which the Institute staff has clear responsibility as regards quality and execution. In the future, it appears this will be of special importance in the areas of international medical research and, under Field Studies, cancer diagnostic test development and environmental cancer. The major program areas which in the near future will receive most attention throughout the Institute are: viruses and cancer; molecular and developmental biology; environmental cancer; and cancer chemotherapy. The highly competitive market for senior scientific personnel in these areas makes it difficult to proceed as rapidly as one might wish, and availability of facilities also sets limits on the rate of development of program segments.

The relationships with the Board of Scientific Counselors continue to be excellent, and the present Board, made up of Drs. Philip P. Cohen, Chairman, Wendell M. Stanley, E. K. Marshall, Jr., Hugh Roland Butt, Jacob Furth and J. Englebert Dunphy, exhibits the same high interest, helpful advice and general support as has become customary. At the April meeting of the Board, a program of research studies by the Clinical Associates was presented by these young men, and at the fall meeting, a program was held on broad aspects of cancer research, its state of development, its future course, and the strategy involved. The Laboratory and Branch Chiefs discussed their programs as they relate to these broad subjects. The fall meeting was the last one for Dr. Wendell Stanley, and it is with regret that the Institute will be without his wise counsel as a Board member. The many valuable contributions he has made to the Institute, not only as a member of the Board, but earlier as a member of the National Advisory Cancer Council and as a consultant on other occasions, is greatly appreciated.

The Board continues to express confidence in the research staff and its programs. The frank discussions characteristic of past meetings continued, and selected problems facing the Institute were discussed with a free exchange of ideas and suggestions. Not only do the members of the Board provide the Laboratory and Branch Chiefs, and, indeed, individual staff members, the opportunity to discuss scientific and organizational problems with them, but as disinterested parties, very real assistance is given to Dr. Endicott and his immediate staff.

The NCI was visited by a delegation of outstanding cancer investigators from the Soviet Union, September 26-28, 1960, in accordance with implementation of the Lacy-Zarubin agreements and subsequent detailed plans developed between the Public Health Service and the Ministry of Health, USSR. The group consisted of:

1. Professor N. N. Blokhin, Dr. med. sci., Director of the Institute of Experimental Pathology and Therapy of Cancer, Moscow, President of the Academy of Medical Sciences, USSR, and Head of the Delegation.

2. Dr. L. A. Zilber, Director, Department of Immunology and Malignant Tumors and Scientific Director, Institute of Epidemiology and Microbiology in the name of Gamalei, Moscow.

3. Dr. L. F. Larionov, Dr. med. sci., Head, Laboratory of Experimental Chemotherapy, Institute of Experimental Pathology and Therapy of Cancer, Moscow, and an editor of "Pathologicheskaya fiziologiya i experimentalnaya terapiya."

4. Dr. M. M. Mayevskiy, Dr. med. sci., Head, Laboratory of Experimental Biotherapy, Institute of Experimental Pathology and Therapy of Cancer, Moscow, and an editor of "Zhurnal mikrobiologii epidemiologii i immunobiologii."

5. V. V. Gorodilova, Candidate in the medical sciences, Head, Laboratory of Virology and Acting Director, Central Institute of Oncology in the name of P. A. Gertzen, Moscow.

Cordial relationships were maintained throughout the visit, and since the main interests of the visitors were virology and cancer, immunology and cancer chemotherapy, most of the presentations by NCI staff members were in these scientific areas. Little new information on cancer research in the Soviet Union was obtained from the visiting scientists. However, two talks were presented: a) Dr. Larionov spoke on "Complex Alkylating Metabolites as a New Type of Antitumor Drug" and Dr. Zilber spoke on "Susceptibility of Rats and Rabbits to Rous Sarcoma Virus." It is planned that a group of American cancer investigators will make a return visit to the Soviet Union in May of 1961. This group will probably consist of Drs. Endicott, Andervont, and Hertz from NCI, and from the Sloan-Kettering Institute, Drs. Horsfall, Stock, Dalldorf and Randall. As part of the program for the exchange of individual scientists who will spend

longer periods of time working in laboratories, Dr. J. Edgcomb may spend six months or longer in the Soviet Union, and two Soviet scientists are scheduled to spend a year at the Sloan-Kettering Institute.

Several members of the staff have received outside recognition for their accomplishments. Dr. Murray Shear is currently President of the American Association for Cancer Research and Chairman of the Finance Committee and of the Chemotherapy Committee of the International Union Against Cancer. Dr. H. L. Stewart is Chairman of the Geographic Pathology Committee of the Union as well as a member of its Executive Committee. Dr. Thelma Dunn is Vice President and President Elect of the American Association for Cancer Research, assuming the office of President next year. Dr. Roy Hertz is Vice President of the Endocrine Society. Dr. Eugene Van Scott is a member of the Board of Directors of the American Academy of Dermatology and Syphilology. Drs. Herbert A. Sober and Carl G. Baker are members of the Executive Committee of the Division of Biological Chemistry, American Chemical Society, and Dr. Baker represents the Division as one of two Divisional Councilors on the American Chemical Society Council. Dr. Harold F. Blum received a Sigma Xi award for his article "Quantitative Aspects of Cancer Induction and Growth: as Illustrated in Carcinogenesis by Ultraviolet Light," American Scientist 47, 250-260 (1959). Drs. John Weisburger and Vincent E. Price became members of the Editorial Board of "Cancer Research." Dr. A. J. Dalton was elected a Director of the Electron Microscope Society of America and was appointed to the Council of the American Association for the Advancement of Science. Many members of the staff continued to give invited lectures and serve in consultative capacities throughout the country.

As an honor to Dr. Wilton Earle and his associates, the Tissue Culture Section was asked by the Cell Collection Coordinating Committee to supply clone NCTC 929 as the first selection to go to the repository for characterized tissue culture cell lines of the American Type Culture Collection.

CLINICAL PROGRAMS

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CLINICAL PROGRAMS

Introduction

The clinical branches of the intramural research effort of the National Cancer Institute have enjoyed a productive year. This is attested to not only by the high quality of research publications on major problems of clinical cancer, but also by continual calls upon the staff for advice, invited lectures and service upon National committees related to cancer research. In addition to the honors listed above, a few others should be mentioned. Dr. Roy Hertz was called upon to advise Secretary Flemming on problems of carcinogenesis. He was also elected to the American College of Physicians. Dr. Nathaniel Berlin was elected to the American Society for Clinical Investigation. Dr. John Fahey spent a year as a visiting scientist at the National Institute for Medical Research, Mill Hill, England. Dr. Mortimer Lipsett is now a visiting scientist at the Karolinska Institute. Dr. Kurt Kohn is working for a period of several years at Harvard with Dr. Paul Doty.

There have been a number of changes among the senior staff. Dr. Donald Watkin, General Medicine Branch, after nine years at NIH, left for a year's work with the Institute of Nutrition of Central America and Panama, surveying nutritional problems of the Caribbean. He will soon assume his new post of Associate Professor of Nutrition at Massachusetts Institute of Technology. Dr. Montague Lane, General Medicine Branch, became Assistant Professor of Pharmacology at Baylor University. Dr. Robert Madden, Surgery Branch, left to become Assistant Professor of Surgery at New York Medical College.

Dr. Thomas Butler, Professor of Pharmacology, University of North Carolina, spent eight months as visiting scientist with Dr. David Rall working on problems of intracellular pH. Dr. Tatuo Saito, Associate Professor of Internal Medicine at Tohoku University Medical School, Sendai, Japan, completed a year as guest worker at the National Cancer Institute. He worked with Dr. David Rall on the transfer of glucose from blood to the central nervous system. Dr. Myron Karon joined the Chemotherapy Service as the first full time pediatrician at NCI. Dr. Paul Carbone transferred from the Hospital Division, to the Chemotherapy Service. Dr. Sherman Weissman, a former clinical associate, returned as a senior staff member of the Metabolism Service. Dr. Jacqueline Whang has accepted a fellowship on the Chemotherapy Service for cytogenetic studies of leukemic patients. The Radiation Branch has Dr. Roger Berry and Dr. Lawrence Draper as new staff members. Dr. Berry is working on the biological effect of neutrons, and on chemical enhancement of irradiation. Dr. Draper's interests concern immunological phenomena as altered by irradiation. Two new senior members have joined the Surgery Branch: Dr. John T. West, who transferred from the Hospital Division, and Dr. Everard Cox, who came from the University of Maryland. In the Endocrinology Branch, Dr. Griff T. Ross has accepted major responsibilities after a number of years at the Mayo Clinic, and Dr. William Odell has joined the laboratory group working on pituitary hormones.

These yearly comings and goings of senior staff raise the questions of what it is that attracts clinical investigators to NIH, why some elect to stay, why some leave. A few remarks might be made about these questions as they relate specifically to the National Cancer Institute. By way of prelude, it may be noted that relatively few senior staff have left during the past year. Such a summary hides completely the real problems. In the first place, three of the four clinical branches have never been able to recruit a full senior staff, so that clinical coverage is extremely thin, with the staff having to spend most of their time on patient care rather than clinical investigation. The situation has been particularly serious in surgery, where a huge service load, inadequate salaries and inability to promote, have discouraged both recruitment and retention. In the second place, the Clinical Director and the Branch Chiefs are seriously concerned that in the immediate future many of our most promising physicians will be lost unless the NIH competitive position is greatly improved. Physicians stay at NIH because the opportunities for clinical investigation have been unique. In spite of relatively low ratio of salary/high-living cost area; in spite of the incredible annoyances of bigness at NIH, such opportunities have thus far held the loyalties of most of our physicians. The steady worsening of personal living standards and the inability to keep within bounds the burdens imposed by NIH size, taken by themselves, might not have constituted a reason for change. However, the recent creation in the university hospitals of clinical resources which for any single investigator could be superior to what he has at NIH, removes the one advantage which we have so far enjoyed. The Branch Chiefs and the Clinical Director of the National Cancer Institute are unanimous in voicing concern about the comparative position of NIH, and are hopeful that the trend can be reversed.

One of the obstacles to full staffing is posed by the nature of the "Cancer Hospital." Some types of research can be justified only in those patients whose disease is not amenable to cure. Thus each patient has relatively far advanced cancer, often with the stresses of pain and physiological impairment, occasionally disfigured from previous surgery, with depleted nutritional and emotional reserves, and death not too far away. One or two of these patients can almost paralyze the usual medical service. Our staff must be prepared to care for over a hundred such patients. To do so is costly -- in resources, in personnel, in demands upon the moral courage of our staff. For such care we must have physicians who are compassionate, skillful, wise and yet good investigators interested in cancer patients. Combinations such as these are uncommon, perhaps rare. There are 8000+ graduates from medical schools in this country each year. NIH receives inquiries from about 1500; 400 are considered seriously; 100 are interviewed by NCI; 18 are appointed as Clinical Associates. From each eighteen in some years, we find one, in some years none, who is qualified to stay on. If we take advantage of the dedicated efforts of these unusual men, do we not have a great responsibility to free them from the gnawing feeling of low standards of living for their families and from the distractions of the red-tape of bigness? I am sure that the Institutes can do much to create a better research atmosphere, such as deliberate "overstaffing" to keep the physicians off the wards at least half the time. Yet

many of the improvements must come from areas not controlled by the Institutes -- higher salaries, more rapid promotion, more progressive personnel, construction, purchasing and travel policies. In fact, much of the adverse feeling of the senior staff about the NIH research atmosphere arises in these very areas. In globo the senior physician and scientist feel that their needs no longer influence the policy decisions on such things as who gets promoted and when, how a job sheet should be written -- and so on.

The content of clinical investigation of cancer requires one comment. No useful purpose would be served by attempting to study all phases of human cancer, just as a century ago it would have been fruitless to arrive at an understanding of virus diseases by studying intensively the mechanisms and control of fever. The leads in cancer research must come from a vigorous non-clinical program. It is the task of the clinician to identify in the clinic those situations which will permit him, in a highly controlled fashion to validate the leads of the laboratory scientist. The clinical program is based on this assumption and the review of the past year's efforts provides many examples. The review will be divided into three sections: Origin of the Cancerous State, Development of Clinical Cancer and Management of Cancer.

I should like to call attention to several developments which can be regarded as of major importance:

1. The description by Dr. Roy Hertz and his colleagues of five-year survivals of patients with metastatic trophoblastic disease following methotrexate treatment.

2. The development by Dr. Eugene Van Scott of ways to study cellular differentiation in man.

3. The definition by Drs. Robert Smith and John T. West of the limitations of sampling of tumor cells in wounds and peripheral blood.

4. The development by Drs. Emil Freireich and Dr. Allan Kliman (DBS) of a practical way to treat thrombopenic hemorrhage by platelet replacement without the development of immunity.

5. The emergence of chemotherapy studies under Drs. Frei, Rall and Goldin into a new phase which allows study of the specificity of animal tumor systems for prediction of drug activity for particular tumors of man.

6. The maturation of the NCI registry of patients to the level that all major studies report 100 percent follow-up.

7. The recognition by Drs. Ketcham, Hoye and Berlin of the magnitude of blood loss during and following surgery, and the failure of the hematocrit to provide a guide for full replacement.

8. The demonstration by Drs. J. R. Andrews and Brace that radiation therapy of some cancers can be safely and adequately given in a single dose.

ORIGINS OF THE CANCEROUS STATE

There is now a large body of opinion which holds that a normal cell becomes a cancer cell because of some damage to the DNA replicating mechanism. This damage can apparently be initiated by a variety of "causal factors" such as irradiation, chemicals, genetic defect, viruses or metabolic abnormality. It has been difficult for the NCI intramural clinician to identify clinical situations worth study. Most of the leads come from epidemiological considerations, and here our clinicians have had few opportunities. Perhaps the newly formed Field Studies Group under Dr. Shimkin will create the openings for the clinician to study carcinogenesis. (One such opportunity developed recently when the uranium miners' study revealed 8 men with suspicious cytology, but lack of adequate surgical staff made admission unwise.)

Another reason for inability to initiate program has been the status of the art of uncovering oncogenic viruses. Drs. Manaker, McLaughlin and Couch studied 27 primary tumors from NCI patients, by tissue culture techniques, designed to unmask such viruses, without positive result. Vigorous pursuit of human oncogenic viruses awaits new discoveries on how to uncover them.

Several illustrations may serve to emphasize how indirect must be the approach of the clinician to carcinogenesis. Drs. Van Scott, Reinertson, Eisen and Rothberg studied patients with psoriasis as a model of skin hyperplasia, and tried to relate the hyperplasia to biochemical abnormality. Recognizing psoriasis as a systemic disease with arthritis and hyperuricemia, they have shown that in this disease C-14 glycine: 1) is over-incorporated into uric acid; and 2) has a much more rapid turnover time (3-5 days) after incorporation into protein of psoriatic scales, than of normal skin (28 days). The reasons for these phenomena are unknown, but the model exists for further study, in psoriasis and in cancer, of the relation of cell growth to disturbances of nucleoprotein and protein metabolism.

The observations of Dr. John L. Fahey on plasma-cell tumors in man provide another possible approach to clinical carcinogenesis. Continued work with Dr. Michael Potter on 8 plasma-cell tumors of mice, showed that the proteins formed by these cells have distinctive electrophoretic, ultracentrifugal and immunologic properties. The individuality of the myeloma proteins suggests that each neoplasm arose from a single plasma cell clone. This observation is in keeping with those of Morris and Van Potter and of

Tomkins on the enzymatic activities of rat hepatomas. Dr. Fahey's work on plasma cell tumors in man shows similar specificity and individuality of proteins and thus the clinician is provided with a marker by means of which the behavior of a malignant cell clone can be inferred. In this manner some of the many new observations on the individuality of chemical activities of induced tumors can perhaps be tested in the clinic.

Another way in which carcinogenic influences in man can be followed is by means of cytogenetic changes. Qualitative and quantitative abnormalities of chromosomes have been described as genetic faults, as a result of radiation and in association with tumors. Drs. Hertz and Ross have used chromosomal counting as a tool for identification of various genetic growth abnormalities in patients which can then be characterized endocrinologically. Dr. Jacqueline Whang has recently joined the staff and is studying cytogenetic abnormalities in the leukemias and in mongolism. In collaboration with Dr. Tjio of NIAMD, she has simplified the methods for chromosomal morphologic study and enumeration, permitting more extensive survey of changes in disease.

DEVELOPMENT OF CLINICAL CANCER

Once established, cancer cells invade and destroy locally, spread into distant tissues and in the process of growth produce varying effects upon the host. The mechanisms of these processes may be studied in the clinic from the following aspects:

1. autonomy of cancer cells;
2. the spread of cancer cells from the point of origin to remote sites, where they become established and grow;
3. the resultant physiologic and biochemical abnormalities.

Autonomy

By autonomy, one means the property of cancer cells by which they no longer respond to those controls which the host normally exerts over cellular proliferation, differentiation, and invasiveness. In the normal situation, for example, the cells of the vermilion border of the lip, although multipotential, remain throughout life as a sharply defined line. Somehow the body relays information to these cells to remain as mucous membrane. Drs. Van Scott and Reinertson have shown that buccal mucous membrane, when transplanted autologously to a skin area, retained its characteristics only when its connective tissue stroma was still attached. When pure mucosa was transplanted subepidermally, it became keratinized epidermis; when placed in the lower corium, it developed duct-like structures. The same pattern was observed after the transplantation of other skin components such as hair roots. These observations have been extended to basal cell carcinoma. Pure basal cell cancer implanted into the lower corium degenerated, keratinized or formed duct-like structures. When transplanted subcutaneously, the tumor always degenerated. The

authors conclude that although the range of responses possible for epithelial cells appears to be a property of the cell line, the specific response is determined by the environment in its local connective tissue stroma. They have further attempted to define the environmental influences leading to keratinization by tissue culture study of epidermal cells. Keratinization is enhanced by the elevation of the pH of the culture medium above normal or the presence of high CO_2 tension. Thus, though the environmental control may be exerted through complex chemical influences, it is also possible that in vivo the control may be physical in nature. Drs. Van Scott and Reinertson have developed a model for the study in man of cellular differentiation of both normal cells and cancer.

Autonomy can also be subjected to clinical scrutiny in the classic situation of the study of hormonal control of sensitive tissues. Dr. Roy Hertz and his colleagues have continued their observations on the mechanisms by which hormones limit or enhance the growth of normal cells and cancer. Drs. Hertz, Griff T. Ross and Mortimer Lipsett in a study of five men, found breast cancer to be remarkably sensitive to orchiectomy. Four of these, after recurrence, responded to estrogen therapy. There were no abnormalities of thyroid, pituitary or adrenal function. Thus they have identified another highly sensitive system for studies of hormonal control. In studies of breast cancer in women, they have found neither exacerbation or regression of disease after the administration of human or sheep follicle stimulating hormone, human growth hormone, or ovine prolactin. The latter two materials caused no exacerbation of prostatic cancer. Growth hormone was studied extensively for its metabolic effects. It did not cause nitrogen retention in four primordial dwarfs. Reports of others on the activity of chymotrypsinogen treated beef growth hormone could not be confirmed. In addition, although testosterone caused nitrogen retention in active acromegaly, growth hormone did not. Dr. J. M. Van Buren, NINDB, in a collaborative report with the late Dr. Delbert Bergenstal, evaluated 12 patients with breast cancer treated by hypophysectomy. Seven of the 12 had remissions lasting 3-9 months. Serial sections of the hypophysis at autopsy indicated the degrees of completeness of pituitary removal. Twenty-nine to 0.01 percent of the gland remained. Neither the degree of depression of endocrinologic function nor the extent of regression of breast cancer were in any way correlated with the residual amount of pituitary. If less than 10 percent of the gland remained, the patients were permanently cortisone dependent. On the other hand pituitary stalk section in another 12 patients with breast cancer showed little effect upon tumor, and several patients showed persistence of thyroid function.

In studies of the hormonal control of prostatic tissue, Dr. William W. Tullner has shown in castrate male rats that ACTH or ACTH plus prolactin produced growth of the ventral prostate only in the presence of the adrenals and hypophysis. He concludes that pituitary growth is, in part, dependent not only on adrenal androgens but also on unidentified pituitary factors.

Spread of cancer

Studies in man by several investigators including Dr. Robert Smith and his colleagues at NCI in collaboration with Dr. R. S. Malmgren and the staff of the Cytodiagnostic Service, have revealed that microscopic fragments and even single cells from a cancer can be found in wound washings after surgery or in the circulating blood. The recent report by Dr. George Moore that ThioTEPA given at the time of radical mastectomy has substantially reduced recurrences compared to controls, emphasize that an understanding of tumor spread is the most important problem facing surgical research. Continued study of cellular detachment, spread and growth in patient and animal during the past year has helped further to define the problem.

Wound washings

Drs. Smith and Marvin Arons have followed 100 percent of 111 patients who had excision of primary cancer between September 1953 and December 1956. The follow-up period since operation was 44 to 83 months. Twenty-six percent of the patients had positive wound washings; 60 percent were negative and 14 percent suspicious. There were no correlations between the results and local recurrence, lymph node or distant metastases, or survival. The lack of prognostic significance of positive wound washings might well be due to sampling problems. Dr. Seymour C. Nash has attempted to obtain more complete wound samples by inserting a suction catheter into the wound for several days. At intervals, samples of the wound washings, trapped and iced in heparin and streptodornase-streptokinase, were removed for counting. Tumor cells were demonstrable in post-operative drainage fluids as long as 43 hours after operation, and sometimes when the washings at the time of operation were free of cells.

Cancer cells in circulating blood

Although other investigators have found no correlation between cells in the circulating blood and prognosis, it is doubtful whether adequate experimental design of a study has yet been attained in man. Dr. John T. West and Dr. Malmgren have continued to validate methodology, and still face problems of identification of cells. An equally serious problem is that of sampling, as shown in their study of 377 patients, 26.3 percent of which were positive for circulating tumor cells. There was a linear relation between the number of examinations and the percentage positive. Of 183 patients with one examination, 10 percent were positive. Percentage positives increased with repeated examinations until, with seven or more examinations, 75 percent of 45 patients were positive. Prognostic significance cannot be judged until sampling comparability is achieved. It can be inferred that the present technique is less than 10 percent efficient. The limiting factor is probably the small amount of blood which can be processed with present techniques. If a method can be found permitting greater efficiency of recovery, then it will be possible to examine the influence of manipulation, exercise, and other factors in releasing cells into the circulation. One modification in the millipore technique has been introduced by Dr. Marvin Romsdahl who substituted dextran sedimentation

for lysis of erythrocytes. This permitted the adaptation of quantitative recovery from blood of the cells of various mouse tumors.

Spread of tumor in animal models

The animal models of tumor spread developed by members of the Surgery Branch have been ably utilized by Dr. Alfred Ketcham and his colleagues. Dr. Romsdahl injected Sarcoma T-241 tumor suspensions into the hind leg of C-57 mice, amputated the left leg on the next day in one group of animals and on succeeding days in other groups. He found that although circulating tumor cells could be found 24 hours after inoculation, amputation before the seventh postoperative day prevented pulmonary metastases. He concluded that the demonstration of tumor cells in blood is not necessarily equivalent to hematogenous metastases.

Last year it was reported by Dr. David Kinsey that certain tumors will metastasize only to pulmonary tissue, even if the lung is transplanted away from the pulmonary circulation. Dr. Marvin Arons found that metastasis did not occur to a similarly implanted etheron sponge in DBA/P mice with L1210 leukemia, S-91 melanoma or a mast cell tumor. Even though connective tissue would rapidly enter the sponge, tumor would grow around but not infiltrate it.

A number of host factors which influence metastases have been recognized. Dr. Ketcham transplanted several mouse tumors into mice of different ages and found that the growth of the primary tumor was delayed in the older mice. Similar delay was noted if the fluid intake of the mice was limited to 20 percent ethanol, but there was no effect from denervation of the limb into which the tumor was transplanted. Dr. Alan Retik showed an increase in metastases of S-91 melanoma to the previously traumatized liver.

Previous reports have emphasized the failure of drugs to modify the local growth or metastases of transplanted tumors. In the past year Dr. Ketcham has shown accentuation of growth of a variety of transplanted mouse tumors when drugs such as clorpactin, thioTEPA or alcohol were placed in the wound before the inoculum. When the order was reversed, there was a delay in growth related entirely to the mechanical effects of washing, except with 3,6-diamino acridine. Dr. Nash reported that with a mast cell tumor, this agent locally applied one to one-and-a-half hours after tumor inoculation reduced takes to 4 percent whereas saline washed controls had 100 percent take. Similar results were obtained with S-91 melanoma. This is an interesting lead because of the variety of acridines available and their pharmacological property of adsorption to nucleoproteins.

EFFECTS OF CANCER UPON THE PATIENT

Clinical cancer has taken the place of syphilis as "The Great Imitator," for it can appear in every conceivable guise. This has always been true but has only recently become fully apparent as the patient with cancer has been recognized as a sick human being, rather than a technical problem in early diagnosis, surgery, radiation or referral to a nursing home. The bewildering kaleidoscope of clinical cancer is in large part due to the capacity of cancer to infiltrate any organ, with resultant change in function, either as a single organ deficit or in unending combinations with damage to other organs, susceptibility to infection and emotional response. The temptation to study intensively these clinical fireworks is difficult for the good physician to resist. And this is fortunate for it leads to precisely the kind of intimate, compassionate and sophisticated care which is the right of every patient and the duty of each physician. It should be appreciated that this "research," vital as it is to the care of the patient and to the continued development of good doctors, contributes little to the understanding of cancer *per se*. This is perhaps not wholly true since some organ destruction of general character, such as of bone marrow in the leukemias or of lymphoid structures in the lymphomas, may relate to the nature of the disease.

In addition to the direct effects of infiltration, cancer produces a number of indirect responses remote from the site of tumor cells. Many of these indirect effects are brought about by chemical substances such as hormones manufactured by the cancer, and even though these do not bear any more relation to the nature of cancer than anatomic and physiologic alterations, they do provide ready-made labels by which tumor behavior can be followed quantitatively. In addition, there are indirect effects of unknown causation, such as nutritional depletion of the host which, if understood, might give clues to the mechanisms of autonomy.

These are the assumptions upon which we have based the clinical investigation of the behavior of cancer.

DIRECT EFFECTS OF CANCER GROWTH

Blood forming tissues -- platelets

The intensive study of mechanism of hemorrhage in the leukemias has continued. It has always been puzzling why some patients with marked thrombocytopenia should show no bleeding tendency and the question has remained as to the need to postulate a pathogenetic factor other than platelet deficiency. Drs. E. J. Freireich, L. R. Schroeder and L. Gaydos have been able to demonstrate in 92 leukemic patients with hemorrhage a direct relationship between degree of thrombocytopenia and risk of hemorrhage. Working in collaboration with Dr. A. Kliman (DBS), a technique has been developed for the separation and concentration of large numbers of platelets. The technique utilizes repetitive plasmapheresis of single

donors. Twenty leukemic children with severe thrombopenia have been treated with these preparations; nineteen showed response with clinically significant increase in circulating platelets and control of hemorrhage. Contrary to expectation, no instance of immunization was seen even though patients received repeated platelet transfusions over a period of several months. The investigators feel that if larger amounts of platelets can be given and thus the treatment made available to adults, the way may be open to the more complete control of thrombocytopenic bleeding.

Dr. Schroeder has continued his search for a "platelet hormone" and has defined some of the conditions for platelet control in rabbits.

Leucocytes and immunity

The effects of diffuse cancer upon leucocytes are quite complex because of the variety of cell types and functions arising in marrow and lymphoid tissues. The interference in leucocyte production is manifested largely by an increased susceptibility to infection. Studies by Drs. Frei, Paul Carbone and Fahey with the collaboration of Dr. J. P. Utz (NIAID) have helped to unravel some of the mechanisms of diminished host resistance. Thus, infections in patients with acute leukemias were shown to be due to rapid depletion of polymorphonuclear leucocytes without alterations in antibody production. Patients with chronic lymphocytic leukemia were shown to have frequent and severe infections which correlated well with inability to respond to antigenic challenge but only partially with the degree of hypogammaglobulinemia. In a controlled comparison, it was shown that prednisone treatment of chronic lymphocytic leukemia increased the frequency and severity of infections, gave no systematic anti-leukemic response and is contraindicated except for management of hemolytic anemia. Susceptibility to infection in other lymphomas seems unrelated to either above factor and is in some way related to inability to combat virus infections, as in seven adults noted with autopsy evidence of cytomegalic inclusion disease.

Dr. Fahey's approaches to the isolation and characterization of antibodies may help to investigate some of the above problems. Working with anti-thyroglobulin and anti-liver nucleoprotein antibodies, he has shown that some antibodies were solely among the 6.6 S gammaglobulins, some were 18 S and some were present in both.

Gastro-intestinal tract

In addition to varied globulin disturbances, hypoalbuminemia is common in patients with cancer. Dr. T. Waldmann, in collaboration with Dr. R. Gordon (NHI), has developed techniques for studying the pathogenesis of serum protein abnormalities. These have been applied to the elucidation of hypoalbuminemic states related to gastrointentir . disease as previously reported. New findings relate to hypoalbuminemia in lymphoma. Study of 15 patients with lymphoma and hypoalbuminemia showed reduction of synthetic rate of albumin in all. Albumin survival was prolonged in two patients with effusions, while survival was shortened in the remaining 13. Albumin loss into the gastrointestinal tract was not observed.

Bone metastases

The behavior of metastases in bone can be a valuable guide to the advance or regression of cancer. Unfortunately, changes in X-ray lag months behind biological behavior. Dr. L. Avioli has used Ca-47 in association with external scanning and metabolic balance studies. He has confirmed the marked elevation of bone formation and resorption in patients with lytic or blastic lesions. The external scanning with Ca-47 seemed to reflect regression or progression months in advance of the corresponding X-ray change.

INDIRECT EFFECTS OF CANCER GROWTH

The production in the host of chemically mediated effects is classically illustrated by endocrine active tumors, myeloma and carcinoid. In addition, there are a number of instances of apparent remote effects where no chemical product has been found and the obverse, in one instance, of a chemical product with no physiological disturbance.

Hormonal products of cancer

The endocrine program of Dr. Roy Hertz and colleagues encompasses many aspects of this problem. The resourcefulness of these investigators is shown in the development of quantitative assay procedures, application to the study of hormonal physiology in animals, and rapid extension to disease problems. Examples of this may be seen in their studies of adrenal cortical hormones. Dr. M. Lipsett, with the collaboration of Dr. H. Wilson (NIAMD), has developed methods for the isolation and quantitation of a wide variety of steroid precursors and end-products. Application of these procedures to patients with functioning adrenal cortical cancer has shown that in all patients there was an elevated excretion of delta-5-pregnenetriol, which percentagewise was greater than the increase in dehydroepiandrosterone. As a result of further analysis of the possible points of enzymatic block, they found that 11-beta-hydroxylation was generally impaired, and in two instances the block was complete. Pregnenetriol was excreted in elevated amounts in many of the patients, both with adrenal cancer and with hyperplasia. They also concluded that 21-hydroxylation was variably impeded and 50 percent of patients with adrenal cancer lacked 3-beta-hydroxydehydrogenase. In studies of the increase in dehydroepiandrosterone, they found that the desmolase could not dispose of the amounts of the precursor, 17-hydroxypregnenolone, presented to it; nor could they show that the increased dehydroepiandrosterone arose from exogenous cholesterol. Excess etiocholanalone excreted by adrenal cancer patients was shown to have unidentified precursors, other than androstenedione and dehydroepiandrosterone.

Drs. Lipsett and L. Garren examined a number of hypermetabolic euthyroidal patients with leukemia or lymphoma. Cortisol was metabolized at a normal or slowed rate, but cortisol secretion was increased. They

concluded that the effect of hyperthyroidism on cortisol metabolism is due to a specific action of thyroxine and is not related to hypermetabolism *per se*. These techniques are also proving helpful in understanding the complexities of endocrine relationships in patients with pituitary tumors, hypophysectomy, genetic growth abnormalities and Cushing's syndrome associated with tumors of non-endocrine origin.

Dr. Hertz has made a number of observations on another chemical product of tumors--chorionic gonadotropin in patients with trophoblastic tumors. In the women with tumor, he found a normal pregnandiol excretion indicating that the luteal phase of ovarian function was unaltered. Four patients with high gonadotropin excretion had normal menstrual cycles, reflecting lack of interference with normal pituitary gonadal relations. Not only does chorionic gonadotropin of pregnancy prolong the luteal phase of the normal cycle, it will stimulate the interstitial cells of the testes of hypogonadal males. In two such boys, the interstitial cells were stimulated by pure gonadotropin from patients with trophoblastic tumors. He concluded that the refractoriness of the ovaries in the tumor-bearing patient involves some special mechanism for interference with peripheral gonadal action. This refractory state appears late in the course of trophoblastic disease; at an earlier phase the ovaries show considerable enlargement.

Another difference between placenta and trophoblastic tumor has been found by Dr. D. Kellogg. Tumor tissue contained only a minute fraction of the steroid dependent transhydrogenase and dehydrogenase found in normal placenta. He also found by histochemical techniques that each enzyme had a different locus in placental cells, supporting the view that they are different enzymes.

Dr. S. Genuth has devised a method for the detection of minute amounts of gonadotropin in the rat hyperemia test. This assay is now applicable to the study of substrate influence on synthesis of gonadotropin by cell free systems.

An annoying fuzziness in gonadotropin assays resulted in findings which illustrate the ability of Dr. Hertz and his colleagues to capitalize on chance, as well as the importance of resolving discrepancies. Unexplained uterine stimulation in immature mice led to the discovery by Drs. Hertz and Tullner that an insecticide, used for ectoparasite control, was responsible. The insecticide proved to be a mixture of Methoxychlor isomers, analogues of the adrenocortical inhibitor, DDD. Further work showed that the mixture was uterotrophic for the mouse, even in the absence of adrenals or anterior pituitary, and that in parabiotic rats it inhibited the gonad stimulating effect of pituitary gonadotropin. It also stimulates testicular growth in male rats, but has little effect on the adrenal cortex of dogs. Further work proceeds with the pure isomers. Thus, alert pursuit of a discrepancy led to a new endocrinologic observation.

Indirect effects on erythrocytes

The mechanisms of anemia in the leukemias and other cancers have been reported previously by Dr. N. Berlin and colleagues. In the past year their attention has turned to tumors and diseases associated with erythropoietin production. Dr. T. Waldmann, in collaboration with Dr. Wendell Rosse, has found an increased mass and rate of synthesis of erythrocytes in patients with cerebellar hemangioblastoma, hypernephroma and pheochromocytoma. Non-dialyzable factors with marked erythropoiesis stimulating activity were isolated from the cystic hemangioblastoma and from the plasma and tumor homogenate of the patient with pheochromocytoma. Erythropoietin was also demonstrated in the urine and plasma of three patients with refractory anemia of unknown etiology. Autopsy in seven such patients revealed hemosiderosis of the liver, an organ which has been suggested as the site of erythropoietin degradation. Dr. Waldmann found that dogs did not destroy erythropoietin during a single passage through the liver. He has also shown in ingenious experiments with parabiotic rats that the kidney is the major but not the sole site of erythropoietic protection. Two other findings of interest in the dog were that cerebellectomy caused no change in red cell mass but administration of desiccated thyroid was associated with a 25 percent increase.

Unexplained indirect effects of cancer

One of the great enigmas of cancer is the mechanism by which the host tissues are depleted during rapid tumor growth. In order to examine this, Dr. Berlin has developed techniques to determine gross body composition and total caloric expenditure. These methods have been applied to three markedly obese patients and nine with various neoplasms. He concluded that measurement of caloric expenditure in man, even with extensive tumor, is feasible but requires thirty days of observation on a metabolic balance regime. Agreement with caloric expenditure values calculated by the Newburgh insensible water loss method, was found in only one-half of the patients.

Dr. Bartter (NHI) and his colleagues have published accounts of a hemodilution syndrome with urinary salt wasting occurring in association with mediastinal tumors, possibly related to inappropriate secretion of antidiuretic hormone (ADH). Drs. Tschudy and E. Hellman have found a similar situation in the crises of acute hepatic porphyria, apparently related to the hypothalamic lesions of this disease. Dr. Tschudy's work on the biochemical lesion of porphyria has led to a finding of interest in animal tumor pathogenesis. As noted previously, the livers of most tumor-bearing animals show a decrease in delta-aminolevulinic acid dehydrase, an enzyme on the pathway of porphyrin biosynthesis. However, a possible exception is found in tumors due to polyoma virus, where no decrease was observed until very late in the course of tumor growth.

Dr. E. McLaughlin has found a marked difference in the biological properties of serum of cancer patients as compared to normals. The method involves the effect of serum on the rate of mitotic activity in the regenerating livers of partially hepatectomized Sprague-Dawley rats. Dr. McLaughlin measured the livers for dry weight, mitotic counts and amount of tritiated thymidine incorporated into DNA. Serum from patients without cancer inhibited liver regeneration by all three measurements. By dry weight measurements, less than 50 percent of the liver regenerated. When serum from patients with cancer was injected, in excess of 70 percent of liver regenerated. This property does not fade when a primary tumor is excised or irradiated. Serum from animals with transplanted tumors inhibited regeneration, while that from animals with spontaneous tumors stimulated regeneration.

MANAGEMENT OF CANCER

As pointed out previously, cancer in man by virtue of its ability to interfere with all bodily functions can induce physiologic infectional or emotional deficits in any combination. The adequate management of cancer must include their early recognition and correction. This report and previous reports have given many examples of the contributions of our clinical investigations to the further definition and control of such distortions of the bodily economy. One instance will suffice to illustrate the role of research in providing rational medical support to the patient with cancer. Drs. Paul Schwab and Fahey have continued their work on Waldenström's macroglobulinemia. As noted last year, the macroglobulinemia is associated with a marked rise in plasma viscosity. Several patients have been treated by repeated plasmaphereses, with reduction in macroglobulins and plasma viscosity. In addition, papilledema, retinal vessel dilitation and hemorrhages, and in one instance, congestive failure receded as the plasma viscosity was reduced.

Although these types of studies are an essential part of the rational management of the cancerous patient, they deal with remote effects and do not necessarily improve ability to control cancer. The modalities available to the clinician are the classical quartet of surgery, radiation, hormonal manipulation and chemotherapy. Endocrinologic aspects have been previously discussed; a few remarks follow on new information gathered in the other areas.

SURGERY

Operative effectiveness

Programs of curative surgery at NCI have been limited to relatively far advanced cancer of the head and neck, and of the uterine cervix. These studies are of sufficient size that it has been possible during the past

year to obtain an estimate of the effectiveness of resection in this unusual type of extensive but still localized cancer.

Drs. Arons and Smith have analyzed the results in 89 patients with cancers of the head and neck, admitted between July 1953 and January 1960. All patients were followed through August 1, 1960, giving a range of follow-up periods of 6-76 months. Life table analyses were made of the 72 patients subjected to definitive surgery. At 2 years, 23% had clinical evidence of distant metastases, and at 3 years, 47% showed local recurrences. Forty percent survival at 60 months was calculated from the life tables.

Dr. Robert Hoye studied the metastatic pattern in the 42 patients who were autopsied. Forty-five percent of these patients had disease limited to a potentially curable site. Fifty-five percent of the patients had metastases below the clavicles; the lung was involved in all but one, but this was apparent ante-mortem in less than half of the patients. This meticulous follow-up in both studies of head and neck cancer patients shows that although distant metastases are perhaps less common than with some other cancers, it could be demonstrated during life in one-fourth of the patients, and at autopsy in one-half.

Drs. Smith and John Dillon similarly reviewed their experience with 131 patients with carcinoma of the cervix, admitted between August 1953 and January 1959. Only 120 of the patients were proven to have uterine cancer, and of these, 117 were epidermoid cancer. Most of the cancers were relatively late; only 3 patients were classifiable as stage I. Management was as follows:

29 patients - clearly inoperable on admission
31 patients - explored and found inoperable
17 patients - radical hysterectomy and node dissection
8 patients - anterior exenteration
35 patients - total pelvic exenteration

Operative mortality for the 60 patients in whom resection was attempted was 2.3%. As of May 1960, 9 of the 17 patients who had hysterectomy, and 17 of the 43 patients who had exenteration, were alive and free of tumor. Of the 34 patients who have died, 9 were free of tumor. In the 7 patients with hysterectomies and recurrence, 6 were local and 2 distant; in the 24 patients with recurrence after exenteration, 12 were local and 18 were distant. Dr. Smith concludes that the surgeon should do more radical surgery in some cases selected for hysterectomy, but avoid it when disease is clinically extensive.

The two studies contain many valuable observations on the natural history of these common cancers, have helped to define the place of radical surgery, and have resulted in the complete acceptance of these procedures by the local medical community.

Complications of radical surgery

Two of the circumstances which sharply limit the safety and completeness of surgery are wound infection and hemorrhage. Studies during the past year by physicians of the Surgery Branch have added vital facts and have provided the means of prevention.

Wound infections

Drs. Jack Bloch and Ketcham found 41 wound infections in 247 operations. Thirty of these were due to Staphylococcus aureus hemolyticus, coagulase positive, and 20 of these occurred in ischemic tissues. Twenty-four of the 30 patients carried staphylococci preoperatively, and where typing was possible, the types were the same -- pre- and postoperatively. There was no evidence for cross-infections among patients, personnel or in the operating suite. A prospective study was then designed, with randomization and coding techniques, in which half the patients received chloromycetin (4.0 grams daily) and half a placebo. There was a 14 percent occurrence of wound infection in 43 chloromycetin treated patients, whereas 52 percent of the placebo treated patients had wound infections. The investigators concluded that 1) wound infections in their patients occurred largely as a result of operating in areas which contain some ischemic tissue and are preoperatively contaminated by staphylococci, and 2) under these conditions, prophylactic chemotherapy is required.

Operative and postoperative blood loss

Although the need for replacement of blood attendant upon surgery is well understood, the use of the hematocrit as the measure of repair may lead to substantial under--or over estimates of amounts required. Drs. Ketcham and Hoye, collaborating with Dr. Berlin, have employed two techniques which allow for precise restoration. The first of these is the use of radioactive chromate labeled erythrocytes for the quantification of total red cell mass. Sixteen patients were studied pre- and post-operatively; blood was replaced as indicated by hematocrit. In three instances the total red cell mass was constant; in three patients there was a net gain of about 250 ml. of red cells. In the other 10 patients there was a failure to replace blood varying up to 800 ml. of red cells. In addition, an occasional patient who had been transfused up to a normal preoperative hematocrit was substantially anemic in terms of red cell mass. In the postoperative period, there was a further decrease in total red cell mass, indicating a loss of 160 to 477 ml. of red cells over 14 days; a loss which could not be recognized grossly or by means of the hematocrit. Thus, the repetitive study of red cell mass, can avoid the dangers related to too many or too few transfusions.

The second technique employed in the operating room has been the utilization of the LaVeen-Rubricus blood loss meter, which by an estimation of blood chloride permits a direct cumulative measure of operative blood loss. Not only did they collect and estimate blood lost by suction, but also the blood on sponges, pads and towels. In 9 patients there was a

370 to 2700 ml. deficit in unreplaced whole blood. In an additional 7 patients, they also measured the blood on drapes and gowns which ranged from 350 to 1465 ml. Further study during the 14 days after surgery showed further reductions in red cell mass. The surgeons concluded that: 1) the hematocrit is an unreliable guide in estimating operative and postoperative blood loss; 2) 15 to 42% of operative blood loss is on the drapes and gowns; 3) following radical surgery, the insidious loss of blood may be substantial, representing 9 to 44% of total red cell mass present immediately postoperatively.

RADIATION

Radiation therapy, while curative of few histologic types of tumor, remains the principal means of providing prolonged regression of non-resectable cancer. Despite its pre-eminence in palliation, it has not been possible to expand seriously its curative potentiality by use of higher energies or better geometry. Tissue culture and animal evidence that chemical modification of the X-ray effect, or the use of other particles such as neutrons, give greater tumor damaging effects, has yet to be put to clinical trial. The program in the Radiation Branch during the past year has in the clinic continued to examine the usefulness of the two million volt external X-ray beam in the management of cancer, either alone or with chemotherapy, and its effects upon bone marrow and renal function. In experimental animals, studies have centered about the physiological processes during recovery from radiation injury and the effect of X-rays upon immune response.

Clinical studies

Drs. J. R. Andrews and K. Brace have long been interested in time as a modifier of the radiation response. Clinical radiation therapy has classically been delivered during a four-to-six-week period. Previous reports have shown that no advantage was apparent when the dose was given during a 100 day schedule. During the past year they have studied the effects at the other end of the scale, namely 2500 r given in a single dose of 40 minutes' duration. Preliminary observations indicate a good tumor response without complications. While it will be impracticable to make a definitive comparison with standard dosage schedules, it should be possible to make some rough estimate of the effectiveness and safety of a single dose. If feasible, such a schedule would radically affect current practice of radiation therapy as well as permit a study of radiation as adjuvant on the day of surgical resection.

Dr. Brace has made several studies of the enhancing effect of chemicals upon radiation responsiveness. He has shown that Actinomycin D, which sensitizes cells in tissue culture to X-rays, similarly increased the radiation response of skin. In three carefully studied patients, however, there was no sensitizing effect of this antibiotic on tumors. Similar observations were made on the enhancing effects of HN_2 upon

radiation therapy of bronchogenic cancer in a collaborative study with the Eastern Group. Disappointingly, neither survival time nor tumor response was improved by the addition of the alkylating agent.

Drs. J. R. Andrews, Brace, J. Levin and Berlin have studied the effects of total body irradiation on erythropoiesis. Patients studied had either disseminated lymphoma or chronic lymphocytic leukemia. They received 85-100 r with 2.5 MEV X-rays uniformly distributed to the entire body. The depression of peripheral counts was profound and reached a maximum for all elements at about 28 days after irradiation. The most sensitive index of bone marrow effect was the plasma iron disappearance rate. It showed maximum depression at two days and a return to normal one week after irradiation. There was also a curious second fall at 28 days. The therapeutic responses obtained from total body irradiation were striking; -- especially in the patients with chronic lymphocytic leukemia, where normal counts were still present six months after therapy.

Drs. L. Avioli and Brace have undertaken a systematic study of the effects of abdominal X-irradiation upon renal clearances. Patients with large X-ray sensitive intra-abdominal tumors have been given 400-500 rad in single doses without consistent change in glomerular filtration rate, renal plasma flow or TmPAH.

Dr. Andrews and his colleagues have continued their studies of the use of S_{35} in the treatment of chondrosarcoma. Two additional patients have been treated during the past year. It is clear that these cartilaginous tumors variably concentrate the S_{35}, presumably in chondroitin sulfate, and that the therapeutic utility is limited by bone marrow depression. Only one of the five patients is still in remission after administration of the radioisotope.

Animal studies

Dr. Roger Berry, who has recently joined the staff, has adapted a method developed in England for in vivo examination of chemical enhancement. He used a lymphocytic leukemia in the ascites form in DBA/2 JN mice. Serial dilutions of the ascitic fluid were injected into groups of mice in sufficient number to obtain a 50 percent take value, thus permitting quantitative estimation of X-ray dose-response characteristics with and without chemical or physical adjuvants. A dose response curve for 3 MEV X-rays has been established. If hydrogen peroxide is given intraperitoneally prior to irradiation, a steeper dose response slope was obtained; the "oxygen enhancement" ratio approximating 2.5:1. In addition, Dr. Berry studied the combined effects of X-rays and 5-bromodeoxyuridine or 5-iododeoxyuridine. Both combinations produced a marked decrease in the number of surviving cells compared to either modality used singly. Using the same system, with the kind cooperation of the staff of the Brookhaven National Laboratory, Dr. Berry has established a dose response curve for fission spectrum fast neutrons.

Dr. W. Smith has continued her survey of the processes involved in spontaneous and induced recovery from irradiation damage. She reports that 1) the time of spontaneous granulocyte recovery varied with age in mice and correlated with ability to survive irradiation, and 2) in granulocyte mobilization studies, the ratio of mobilized to non-mobilized cells was similar for irradiated and normal mice. Endotoxin injection, which improves survivorship, caused a fourfold increase in granulocytes.

Dr. F. Smith has studied the effect of irradiation upon the ability of mice to form hemolysin to sheep erythrocytes. Irradiation diminished antibody production and this was a function of total dose rather than rate administration of X-rays. In rats, he found that altitude acclimatization increased capacity to form antibody hemolysin. Dr. Smith has also studied immune responses in C_3H mice with the plasma cell tumor X-5563, which were given X-irradiation and specific antibody. Return of the immune response to near normal limits was observed in the tumor-bearing mice immunized as early as 24 hours after irradiation.

Dr. C. Maxwell in his long-term studies of the effects of irradiation of amino acids has added a thirteenth compound, glycolic acid, to the list of materials appearing when solutions of glycine are irradiated with X-rays. Dr. P. Riesz has evidence for two kinds of hydrogen atoms differing in chemical reactivity which appear in the abstraction reactions resulting from X-irradiation of water. Dr. H. Andrews found that mice can sense an X-ray beam under some circumstances. This is probably related to ozone production. He has also developed dose response data for swine from the mid-lethal range to several thousand r. Offspring from heavily irradiated parents had a dose response curve identical to those of the parents.

Drs. M. Kelly, R. W. O'Gara and T. L. Loo have continued their studies of chemical protection against X-rays and radiomimetic drugs. Previous studies have shown that amino-ethylisothiuronium (AET) protects against both and that those tissues which highly localize the drug receive the most protection. C_{14} labelled AET has been used to study its intracellular localization by microautoradiograms. It was found distributed uniformly throughout the cytoplasm, without intranuclear incorporation. Compounds which generate free radicals have been found to be radioprotective. Di-t-butyl peroxide or t-butyl perbenzoate was injected intraperitoneally in mice or rats 30 minutes before HN_2 or whole body X-irradiation. These agents reduced the lethal effectiveness of HN_2 or X-ray by about 50%. In studies with Dunning leukemia, neither drug interfered with the therapeutic effectiveness of HN_2.

General Remarks

Cancer chemotherapy has reached a new phase, and it is time to adjust attitudes and planning to this perspective. From the observations of Dr. Hertz and his colleagues on trophoblastic tumors, it is clear that a drug can induce at least 5-year survival of patients with cancer. This means that a drug, in one type of cancer, not only assumes a place with surgery and radiation as primary therapy for cancer, but also can accomplish what surgery and radiation could never attempt, the control of diffuse metastatic disease. One problem then is the exploitation of this success, to identify the screening system which could have predicted it. This concept should be extended to the whole search for chemical control of cancer -- namely, the task of proving the value of a biological system which can predict drug responsiveness for each of the many diseases known as cancer. Five years ago there was confusion in drug screening because of the number of variables and the lack of quantitative data for either man or animal. Due to the efforts of CCNSC, the cooperation of scientists throughout the country, and I believe the leadership of several NCI scientists and physicians, quantitative data have been gathered and the beginnings of such screening correlates are within our grasp. Predictability seems highly probable for acute leukemia, possible for choriocarcinoma, and other screens, both transplanted animal tumor and tissue culture, are partly categorized for the compounds they find or miss. Finally, the easy convenience of transplanted rodent tumors has been in part abandoned for exploration of the more difficult screening techniques of spontaneous tumors and those induced by viruses, carcinogens and radiation. For the above reasons, it is possible to abandon in large part any random search for the panacea, and to focus on particular cancers. This means that the start must be made with man and that the basis for establishment of screening specificity should be the chemicals which control specific cancers in man.

The intramural chemotherapy program of NCI

The intramural program has been developed on the assumption that it was an integral part of the National effort in cancer chemotherapy, and yet had resources and interests of its own. It was felt that thus our scientists could actively share work load and data with others similarly motivated, and still be able to pursue imaginatively their own research ideas. Since it was undesirable to duplicate expensive facilities, the intramural group developed no synthetic program or primary screen of its own, but depended entirely upon CCNSC. As its share, the intramural program pioneered with quantitative screening, developed data on toxicity and pharmacology of new agents and was responsible for the leadership of two of the clinical groups. The ground to be covered was so vast, the objectives so uncertain, that several years of trial and error were required to develop rational methodology. During the past 5 years many of these methodologic puzzles have been solved, and the intramural program

is ready to plan the next phase, that of utilization of calibrated techniques for the uncovering of chemical control of some specific cancers.

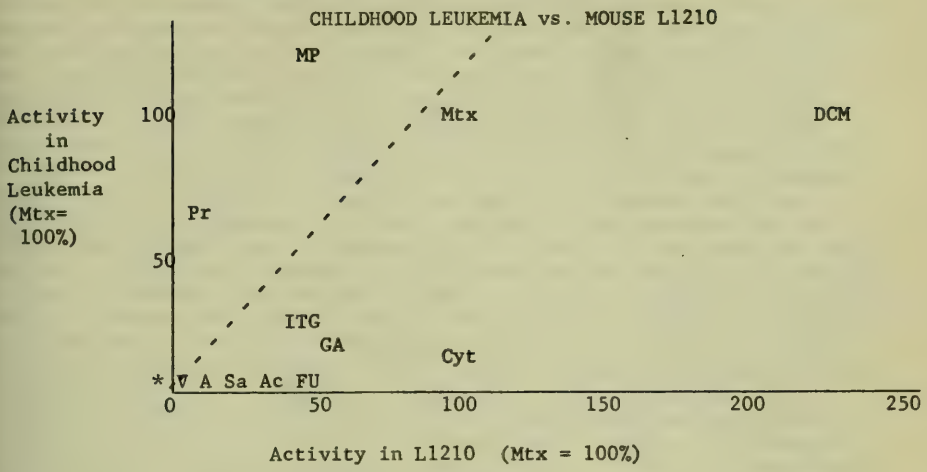
Specific anti-tumor agents

Antifolic compounds

Drugs with antifolic activity were among the first anti-tumor agents to be discovered. Synthesis of these compounds was extraordinarily difficult, and after the first few compounds failed to show increased activity in microbiological systems, chemists turned with relief to the newest drug, 6-mercaptopurine. Despite the ease of synthesis of the anti-purines, 6-mercaptopurine has proved of narrow utility. Methotrexate, an antifolic, not only induces complete remission in acute lymphocytic leukemia and metastatic trophoblastic tumors, but has been shown by Dr. Van Scott to cause regression of basal cell cancer, and by Dr. Frei and his colleagues to cause regression in several lymphomas. NCI, thanks to CCNSC and intramural joint efforts, has been able to undertake a broad structure-activity synthesis program with Southern Research Institute.

One of the reasons for the interest in antifolic synthesis, has been the discovery by Dr. Goldin and colleagues of the increased activity of antifolics brought about by halogenation in positions 5 and 6. As previously reported, he found in advanced L1210 leukemia in mice that dichloromethotrexate or bromochloro-methotrexate as compared to methotrexate, effected a threefold increase in survival time. This quantitative screen has developed into a predictability system of considerable value for acute lymphocytic leukemia as shown in the accompanying chart developed by Drs. Frei and Goldin:

COMPARISON OF THERAPEUTIC INDICES**



*Azauracil, Nitrosoguanidine, Narcotine,
V=Vincaleucoblastin, A=Actinomycin D, Sa=Sarcolysin, A=Azauridine,
FU=Flourouracil, Pr=Prednisone, MP=6-Mercaptopurine,
Mtx=Methotrexate, ITG=Imidazolylthioguanine, GA=Guanylhydrazone,
Cyt=C ytoxan, DCM=Dichloromethotrexate

**The rational use of animal screens as predictability systems for cancer in man requires quantitative data such as these. It has taken 5 years of intensive effort to collect data for this figure. Note that it involves one animal tumor and a single disease. A similar figure cannot at this time be constructed for any other cancer in man. The magnitude of finding curative drugs for several cancers in man should not be underestimated.

As a working hypothesis, those compounds with greater than 50 percent of the activity of methotrexate can be expected to be active in acute lymphocytic leukemia. It has been disappointing to recognize that the most active compounds yet found, the halogenated antifolics, have not been similarly more effective in leukemia. Dr. Frei and colleagues, working with Leukemia Group B, found that oral dichloromethotrexate was no more active than methotrexate in acute lymphocytic leukemia. The reason for this was thought located when Dr. Goldin reported that in L1210 oral administration of dichloromethotrexate was associated with sharp loss in therapeutic effectiveness. Repetition of the clinical study by Leukemia Group B, using parenteral drug, did not appear to increase activity in acute lymphocytic leukemia. Intensive studies of the pharmacology of dichloromethotrexate by Drs. Oliverio, T. L. Loo, J. D. Davidson, D. Rall and Mr. R. Dion, have provided a clue, though perhaps not the full solution, to the discrepancy. By the use of DEAE ion-exchange columns, they were able to separate and quantify various folic acid analogues and found that 40-50 percent of dichloromethotrexate was excreted in the urine in the 24 hours following a parenteral dose. Moreover, one-fifth of the dichloromethotrexate was in the form of a metabolite, characterized as the 4:7-dihydroxy derivative. They also synthesized dichloromethotrexate containing radioactive chlorine, and found that it was rapidly excreted in the bile of rats, mice, dogs and man. Since it is poorly absorbed from the intestine, biliary excretion effectively removes the drug from the blood. The parent compound because of these two processes rapidly lost its effectiveness. This does not explain the discrepancy with the screening animal, since qualitatively at least the pharmacological differences between mouse and man are not great.

Drs. Hertz, Lipsett, Ross and colleagues have now treated 68 women suffering from trophoblastic disease with antifolics. Forty-five percent of the patients are in complete remission after methotrexate treatment. The first few patients treated have sustained their remissions for over 5 years. The possibility that the therapeutic response is related to maternal immunization by fetal tissues cannot be supported. In the first place, methotrexate seriously interferes with many immune phenomena. Secondly, Dr. Hertz in a study of red cells and plasma from 35 couples in the above series, could find no evidence of maternal immunization. The patients who have relapsed have been refractory to nitrogen mustard, cytoxan and actinomycin D. However, 6 of 14 methotrexate-resistant patients have responded to an alkaloid, vincaleukoblastine. Two of these have remained in complete remission for 8 and 10 months respectively. The manner in which this compound was selected is highly pertinent to the previous discussion of screening. Dr. Hertz has been able to establish heterologous growth of human choriocarcinoma in the cheek pouch of the untreated hamster in 6 of 35 attempts. Some of these were established prior to treatment and are methotrexate-sensitive. Others are methotrexate-resistant and are used to screen potential anti-trophoblastic drugs. Two active compounds have been found, vincaleukoblastine and podophyllotoxin, and it is of interest that neither has activity in the L1210 screen or in acute lymphocytic leukemia. Thus these two methotrexate-sensitive tumors — trophoblastic disease and acute lymphocytic leukemia will likely have entirely different screens for predicting active drugs.

Purines and pyrimidine antagonists

The discovery that 6-mercaptopurine was an effective drug in the leukemias brought a new measure of hope for the chemical control of these diseases. Not only was 6-mercaptopurine less toxic than the antifolics, it had activity in the myelocytic leukemias, both acute and chronic, as well as in the acute lymphocytic form. Dr. Frei and his colleagues in collaboration with Leukemia Group B have completed their definitive comparisons of 6-mercaptopurine and methotrexate, as reported last year. They have gone forward in their methodologic studies to examine the usefulness of sequential design in 6-mercaptopurine treated acute leukemias. All previously untreated children with either type of acute leukemia were given prednisone. When remission occurred the patients were placed on continuing 6-mercaptopurine or placebo. The duration of remission was used as the measure of effect and it was quickly shown that a small number of patients could demonstrate the active drug. Moreover this design, since it incorporates initial therapy with steroids, permits the use of new drugs in the study of untreated patients. The safety of this maneuver is further reinforced by the fact that relapse after steroids plus new drug can be fully treated with 6-mercaptopurine or methotrexate. Finally, it has the further advantage that new drugs can be given while the patients are in steroid remission, and there is little danger of confusing toxicity with the complications of acute leukemia.

The screen which is best for indicating antipurine type of activity is CA 755. Dr. Goldin and his colleagues have developed this system into a reliable quantitative measure of comparative activity of thio-purines. The only compound of greater activity than 6-mercaptopurine has been its riboside. Preliminary studies in man indicate little advantage, but the definitive comparison is in the future. Imidazolyl thioguanine, which is somewhat less effective against 755, had less activity than 6-mercaptopurine in 52 patients with acute leukemia treated by Leukemia Group B. Recently, CCNSC held a conference about the thiopurines in which Drs. Goldin and Frei participated. There was general agreement to limit the synthesis of these compounds until 6-mercaptopurine riboside activity in man is fully explored, and more is known about the effect of 9-ethyl, and 9-butyl-6-mercaptopurine in man.

In spite of the failure of the synthesis efforts to produce better drugs, 6-mercaptopurine has provided an excellent model for biochemical studies of resistance. Dr. Davidson has previously reported studies of L1210 leukemia in which resistant leukemic cells showed reduction in inosinic phosphorylase. Studies in the past year with the isolated enzyme have confirmed the cellular findings of competition between 6-mercaptopurine and hypoxanthine for inosinic phosphorylase. Dr. Davidson also has developed new techniques for the assay of the enzyme in human leukemic cells. Eighteen patients have been studied and all showed some ability to convert hypoxanthine C_{14} and 6-mercaptopurine S_{35} to nucleotides. Four apparently 6-mercaptopurine resistant patients have been studied and two of these had normal amounts of the enzyme, suggesting that their resistance was not the same as that of mouse leukemia L1210.

Other studies of leukemic leucocytes with a pyrimidine antagonist, azauridine, have been carried out by Dr. H. Fallon. Azauridine is known to inhibit orotidylic decarboxylase, essential for pyrimidine biosynthesis. Dr. Fallon has found that the enzyme in leukemic leucocytes is profoundly inhibited by drug. Azauridine causes a sharp fall in the peripheral leucocyte count of leukemic patients and this correlates well with enzyme inhibition.

Finally, intrathecal 6-mercaptopurine riboside is being used for the treatment of leukemia involving the central nervous system. As reported last year, central nervous system leukemia is occurring with high frequency and is a common cause of treatment failure. This is largely because of failure of methotrexate or 6-mercaptopurine to achieve therapeutic concentration in the central nervous system. Dr. Rall and colleagues have continued their studies of the principles of drug transfer from blood to the central nervous system. Working both with dogs and dogfish, they have evidence that the failure of acids, such as sulfanilic and para-amino hippuric, to achieve high concentrations, in spite of continuous high blood concentrations, is due to bulk out-flow of cerebrospinal fluid (CSF), removing fluid and drug at such a rate that inward transfer rates are inadequate to reach a ratio of unity. Drs. Rall and Streicher (NIMH) have for the first time found a compound, thiocyanate, which has a variable CSF/plasma ratio related to plasma concentrations. At plasma thiocyanate concentrations of 8-10 mM/L, the ratio approaches unity, while at 1-2 mM/L the ratio is 0.1. They suggest that this is evidence that the bulk return of CSF into blood is an active process.

Binding to proteins limits passage of drugs into the CSF. Dr. Rall and his colleagues, in studying the protein binding in various species, have noted the absence of albumin in the dogfish with predictable effects on central nervous system transfer of drugs. They also report the absence of albumin from the plasma of sea lamprey larvae (P. marinus dosatus). Associated with metamorphosis into the adult, there appears a new plasma protein, with electrophoretic characteristics different from mammalian albumin.

Alkylating agents.

The first non-hormonal drug shown to affect disseminated cancer was nitrogen mustard. It clearly can cause substantial regression of Hodgkin's disease and other lymphomas, both forms of chronic leukemia, mycosis fungoides, neuroblastoma and adenocarcinoma of the ovary. It has erratic and insubstantial effects in other epithelial tumors and none in the acute leukemias. In the 15 years since the discovery of nitrogen mustard, many other alkylating agents have been synthesized, shown to have activity in a variety of transplanted tumors and a few put into clinical trial. The programs of NCI, both of CCNSC and the intramural area, have undertaken considerable research concerning alkylating agents. Both groups have participated in recent conferences attempting to interpret the vast amounts of information. It is clear that alkylating agents vary considerably in quantitative and qualitative effectiveness in transplanted animal

tumors. There is some evidence that this is also true in man -- for example: 1) Myleran has much greater activity in chronic myelocytic leukemia than in any other disease; 2) Cytoxan has a modest degree of activity in acute leukemia, whereas the other alkylating agents do not; 3) Cytoxan has relatively little effect on megakaryocytes and platelets as compared to other alkylating agents. Nevertheless the degree of difference found in transplanted animal tumors is not generally reflected in man. I believe the difficulty may represent in an outstanding way what has been said above about screening. The guiding principle of the past has been to find a generally active drug in animal tumors which will be generally active in human cancer. What is needed, before the lead of the alkylating agents is abandoned, is specificity -- particular animal systems which can dependably predict activity in a single human cancer. We have nowhere in alkylating agent research approached the knowledge we have about L1210 and acute lymphocytic leukemia and Ca 755 and myelocytic leukemia.

NCI intramural scientists are participating in several efforts to find such specificity, using transplanted rodent tumors and induced tumors. With the former, Dr. Goldin has attempted to develop Sarcoma 37 into a quantitative screen. This tumor has been unaffected by all the standard drugs, and it was hoped that it would be an effective screen for alkylating agents of high potency. Recently cytoxan and alanine mustard have been shown to be active, but the meaning of this in the clinic has yet to be determined.

Dr. Leon Schmidt of Cincinnati has undertaken a thorough study of the value of rat transplanted tumors in predicting drugs for specific cancers. He has found that relatively slight alterations in experimental design can change markedly the relative activity of an alkylating agent. With control over these experimental variables, he is now able to rank various major alkylating agents for order of effectiveness and is cooperating with clinicians and CCNSC in trying to relate these to clinical predictability. Because of the mass of data, this will probably be solved only with the help of computers. Dr. A. Pratt of the Physiology Laboratory and several NCI clinicians have worked with Dr. Schmidt in developing mathematical models suitable for the examination of this problem. When more clinical information is available on newer alkylating agents, the specificity (or the lack of it) of these rat systems can be understood for the first time.

Since it is possible that transplanted animal tumors may not provide the required specificity, non-transplanted tumor systems are being developed. Dr. Goldin and his colleagues have developed the Moloney leukemia virus into a partially quantitative system. They have also been attempting to use similarly the spontaneous breast tumors arising in the mouse colony at NIH. In both instances preliminary results with drugs warrant the development of larger supplies of these tumor types.

Drs. M. Kelly and R. O'Gara have been studying drug effects in carcinogen-induced tumors. Chemical carcinogens were given to new-born mice; they developed pulmonary tumors, leukemia and fibrosarcomas. Drug

therapy was given from age of 8 weeks to 16 weeks, and the frequency of pulmonary tumor nodules noted. In preliminary observations alkylating agents or cortisone had more effect than antimetabolites.

o,p' DDD (2,2-bis(2-chlorophenyl-4-chlorophenyl)-1-1-dichloroethane).

Dr. Hertz and his colleagues have continued their studies of the effect of this adrenal cortical inhibitor upon adrenal cortical cancer. Eighteen patients have been treated and all but 4 have shown depression of tumor hormone output. Seven patients have had regression of metastatic disease and clinical improvement. Damage to normal human adrenals has also been demonstrated. Pharmacologic studies have shown that much of the drug appears in the stool after oral administration. Nevertheless infusions of the drug in Lipomul did not increase activity. The drug is concentrated in fat depots which rapidly remove circulating compound.

This compound is of interest because it was discovered without the aid of an animal tumor screen, since to date it has not caused regression of animal tumors. Since it does not affect normal adrenal activity of rodents, "screening" for activity of analogues is carried out in the dog, whose normal adrenal cortex is highly sensitive. Dr. W. Tullner has found that duration of treatment and drug dose of o,p' DDD was correlated with degree of cellular destruction in the male dog. He prepared animals in which damage was confined to zona reticularis and zona fasciculata with the zona glomerulosa appearing normal. After cessation of drug, the adrenal secretion of 17-hydroxysteroids was reduced to extremely low levels. Gradual recovery of hormonal output and normal histology appeared in 4 weeks and autopsy showed regeneration of zona fasciculata. Dr. Tullner concludes that the zona glomerulosa is multipotential and even though it produces aldosterone and does not contain the enzymes for hydrocortisone production, it can give rise to fasciculata cells which can carry out this hydroxylation. He has also shown that intravenous difluoro, dinitro DDD was as active in the dog as o,p' DDD, but possessed no advantage over that compound.

CONCLUSION

In conclusion, I shall indicate several areas where resources not now extant are required. Additional laboratory space in the Clinical Center is clearly inferred from the need to have deeper staffing. An "Operations Research" group is required to consider in a formal way clinical data management--its collection, coding, analysis--and the development of mathematical models for computational examination. Several areas are in need of primate breeding facilities for carcinogenesis studies in the newborn as well as for trophoblastic tumor pathogenesis. Resources are in demand for large mouse colonies for spontaneous tumors and for special isolation facilities for chemotherapy and other studies of virus-induced tumors. Personnel and facilities should be planned for a systematic examination of predictability value of animal systems for toxicity of drugs for man. In this regard electron microscopy of drug damaged structures

will certainly be needed as light microscopy is non-specific for such pathology. Finally, personnel and resources should be planned for the expansion of clinical cytogenetics.

I should like to extend to the Director, NCI, and Director, Clinical Center, and their staffs my gratitude for the kind and effective manner in which they have met the mercurial demands of the clinical program. May I also express my appreciation to Dr. Stuart Sessoms and his staff for their extraordinary help in working with us in the chemotherapy program. I want to make known my thanks to the Laboratory Chiefs and their staffs for their kindly help to the clinicians. I am grateful to Mr. Walter Magruder, Mrs. Martha Rees and the secretaries of OADR for their cheerful help in carrying out the functions of my office. Finally, it is a pleasure to acknowledge my indebtedness and thanks to the Clinical Branch Chiefs and all their physicians and to Miss Burgess and Mrs. Kilian and their staffs for courteous support in many ways, but especially in bringing to NCI patients the superb medical care which is the beginning of all clinical investigation.

Respectfully submitted,

C. Gordon Zubrod, M. D.
Clinical Director
National Cancer Institute

January 24, 1961

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Serial No. NCI-15-000 (c)
1. NCI
2. OADR
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Screening

Principal Investigator: Dr. Charles G. Zubrod

Other Investigators: Physicians participating in patient care

Cooperating Units: General Medicine Branch, Surgery Branch, Endocrinology Branch, and Radiation Branch

Man Years:
Total:
Professional:
Other:

Project Description: This is a mechanism by which this necessary hospital function can be classified.

Part B included Yes / / No /X/

Serial No. NCI-15-001 (c)
1. NCI
2. OADR
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Service for other Institutes

Principal Investigator: Dr. Charles G. Zubrod

Other Investigators: Physicians participating in patient care

Cooperating Units: General Medicine Branch, Surgery Branch, Endocrinology Branch, and Radiation Branch

Man Years:
Total:
Professional:
Other:

Project Description: This is a mechanism by which this necessary hospital function can be classified

Part B included Yes / / No /X/

Serial No. NCI-15-002 (c)
1. NCI
2. OADR
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Emergency Admissions

Principal Investigator: Dr. Charles G. Zubrod

Other Investigators: Physicians participating in patient care

Cooperating Units: General Medicine Branch, Surgery Branch, Endocrinology Branch, and Radiation Branch

Man Years:
Total:
Professional:
Other:

Project Description: This is a mechanism by which this necessary hospital function can be classified.

Part B included Yes / / No /X/

Serial No. NCI-800(C)
1. Endocrinology Branch
2. Endocrinology Service
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Endocrine Aspects of the Progression and Therapy of Cancer of the Breast in Women and Men.

Principal Investigators: Dr. Roy Hertz, Dr. M. B. Lipsett and Dr. Griff T. Ross

Other Investigators: Dr. John Lewis, Dr. Howard Schwartz, Dr. Saul Genuth, Dr. Martin Nydick, Dr. Leo Stolbach, Dr. Benjamin Borowsky and Mr. Silas Jackson

Cooperating Units: Dr. J. Van Buren, Neurosurgery and Dr. Robert Smith, Surgery Branch.

Man Years (calendar year 1960):
Total: 13
Professional: 8
Other: 5

Patient Days (calendar year 1960):
Patients:
Days:

Project Description:

Objectives: 1. To extend and intensify detailed clinical observation of the natural course of cancer of the breast in men and women in order to better appraise the effectiveness of various methods of therapy and learn more concerning the etiology and pathogenesis of the disease.

2. To improve upon existing forms of hormonal therapy for palliation of advanced breast cancer and to elucidate the mechanisms involved.

Methods Employed: 1. The acceptance of complete clinical responsibility for the further management of referred patients with proven diagnosis of cancer of the breast and the application to their problem of all available modes of therapy.

2. The exploration of new modes of hormonal therapy for palliation.

3. The detailed analysis of endocrine and metabolic factors operating in relation to the genesis and course of the disease in these patients up to the time of their demise.

4. Careful appraisal of pathological specimens obtained at biopsy or autopsy in collaboration with Pathology staff.

Major Findings.

1. Five men with primary cancer of the breast have been studied from both the hormonal and therapeutic point of view. The history of each patient revealed no endocrinopathic background. Their post-natal, pediatric, pubertal and reproductive histories were all normal. They all showed normal testicular and genital development and the secondary sex characters were well developed in each patient. Their steroid excretion pattern as revealed by urinary ketosteroid and corticoid levels was normal. Studies of thyroid and pituitary function revealed no abnormalities. Thus no associated endocrine abnormality could be related to the unusual manifestation of breast cancer in the male.

These patients all exhibited remarkable regression of disease following orchiectomy, thus bespeaking the hormonal dependency of their tumors despite the apparent absence of any endocrinopathic pattern. Four of them subsequently responded to massive estrogen therapy, again indicating further hormonal sensitivity of male breast cancer.

2. Five women with breast cancer have been treated with sheep Follicle Stimulating Hormone (SFSH) prior to ovariectomy. Although three of them showed substantial ovarian stimulation there was no clear indication of exacerbation of disease in terms of pain, calcium excretion or x-ray findings. Three women similarly treated with Human F.S.H. (HFSH) also exhibited distinct ovarian stimulation unassociated with progression or exacerbation of disease.

3. Attempts at surgical hypophysectomy in breast cancer patients have led to varying degrees of completeness of hypophysectomy, as determined by subsequent study of serial sections of the sellae of these patients. The relationship of residual pituitary tissue to endocrinological status and clinical response was determined and correlations and discrepancies were noted from case to case. (Dr. Bergenstal and Dr. J. Van Buren).

4. (a) Neither human growth hormone nor ovine prolactin exacerbated human breast or prostate cancer. It was shown that the increase in urinary calcium excretion following growth hormone does not depend on stimulation of osteolytic metastases.

(b) The metabolic effects of human growth hormone were examined in a variety of patients and its spectrum of activity outlined.

(c) Growth hormone did not cause nitrogen retention in 4 primordial dwarfs although 3 normal children responded with nitrogen retention. This suggests that at least some of the primordial dwarfs may be an example of end-organ unresponsiveness.

(d) Growth hormone did not cause nitrogen retention in active acromegaly although testosterone did. Thus, either the mechanism of anabolism or the sites of protein deposition differ with the two anabolic agents.

(e) We have been unable to confirm our previous report of an anabolic

Major Findings (con't.)

effect of ovine prolactin. The reasons for the nitrogen retention observed in the first 4 cases are not clear.

(f) In a large series of metabolic studies, we could not confirm reports by others that a chymotrypsinized beef growth hormone preparation was active in man.

(g) In collaboration with Dr. Julius White of the Laboratory of Physiology, NCI, we have completed a study of the parameters of protein metabolism using N^{15}-glycine. Growth hormone and testosterone differed in their mechanisms of nitrogen retention (Dr. Lipsett).

5. A series of 12 patients with breast cancer, subjected to hypophyseal stalk section, has been closed. It is apparent that the effects on the cancer were not as those of hypophysectomy. The endocrine effects were of interest, since several patients showed persistence of the thyroid-pituitary axis with normal thyroid function (Dr. Lipsett).

6. The first documented case of post-varicella hypothalamic disease with secondary hypothyroidism has been studied (Dr. Lipsett).

7. The enzyme leucine amino-peptidase is not elevated in the blood of choriocarcinoma patients as it is in late pregnancy (Dr. Lewis).

Significance to the Program of the Institute: The direct pertinence of the foregoing studies to the grave problem of advanced or persistent breast cancer is too obvious to require discussion.

Proposed Course of Project: The further course of these studies are to be essentially along the lines outlined above. Emphasis is to be placed on the endocrine aspects of breast cancer in the male.

Part B included.

Attachment I
Serial No. NCI-800(C)
Endocrinology Branch

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

1. Lipsett, M. B. and Bergenstal, D. M.: Lack of Effect of Human Growth Hormone and Ovine Prolactin on Cancer in Man. Cancer Res. 20: 1172-1178, 1960.

2. Lipsett, M. B.: Comparison of Metabolic Effects of High and Massive Doses of Prednisone. J. Lab. & Clin. Med. 56: 63-71, 1960.

3. Lipsett, M. B., Engel, H. R. and Bergenstal, D. M.: Effects of Glucagon on Plasma Unesterified Fatty Acids and in Nitrogen Metabolism. J. Lab. & Clin. Med. 56: 342-354, 1960.

4. Wilson, H., Lipsett, M. B. and Butler, L. C.: Steroid Excretion in Hypophysectomized Women, and the Initial Effects of Corticotropin: A Study in Urinary Steroid Patterns. J. Clin. Endocrinol. & Metab. 20: 534-545, 1960.

5. Van Buren, J. and Bergenstal, D. M.: An Evaluation of Graded Hypophysectomy in Man. A Quantitative, Functional and Anatomical Study. Cancer 13: 155-171, 1960.

6. Lipsett, M. B., Moy, R. H. and Bergenstal, D. M.: A Study of Some Metabolic Effects and Urinary Metabolite of 6-alpha-methyl-17-alpha-acetoxyprogesterone. J. Clin. Endocrinol. & Metab. (to appear).

7. Lipsett, M. B. and Bergenstal, D. M.: Metabolic Effects of Human Growth Hormone and Growth Hormone of Other Species in Man. J. Clin. Endocrinol. & Metab. (to appear).

Honors and Awards Relating to this Project:

Dr. Roy Hertz

Elected member of Editorial Advisory Board of Voice of Medicine, Excerpta Medica, Amsterdam.

Fellow, American College of Physicians, San Francisco, April 1960.

Invited to speak before the Roanoke Academy of Medicine on December 5, 1960.

Part B (con't.)

Participated in course on Chemotherapy of Solid Tumors, Madison, Wisconsin, August 25-27, 1960.

Invited to speak to Staff of Johns Hopkins Hospital, Baltimore, Md., March 5, 1960.

Dr. M. B. Lipsett

Invited to lecture at Was hington Hospital Center.

Serial No. NCI-803(C)
1. Endocrinology Branch
2. Endocrinology Service
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: The Nature of Hormone-Producing Tumors of Pituitary, Adrenal, Ovary, Testis, Pancreas, Parathyroid and Chorion; Abnormalities of Somatic Development and Growth.

Principal Investigators: Dr. Roy Hertz, Dr. M. B. Lipsett, Dr. Griff T. Ross and Dr. William Odell

Other Investigators: Dr. Howard Schwartz, Dr. Saul Genuth, Dr. John Lewis, Dr. Martin Nydick, Dr. Benjamin Borowsky, Dr. Leo Stolbach, Mr. Howard Erwin, Mr. Silas Jackson, Mr. Philip Rayford and Mr. Jon Porter, Mr. Frederick G. Dhyse

Cooperating Units: Dr. R. Bates, Dr. H. Wilson, Dr. J. H. Tjio, NIAMD; Dr. L. Thomas, Dr. J. Edgcomb, Path. Anat., NCI; Dr. Robert Smith, Surgery Branch, NCI; Dr. Paul Schmidt, DBS and Miss Alice Stang, CC, Nutrition.

Man Years (Calendar year 1960):
Total: 26
Professional: 21
Other: 5

Patient Days (calendar year 1960)
Patients
Days

Project Description:

Objectives: 1. To learn more about the basic metabolic phenomena involved in the process whereby neoplasms in hormone-producing organs produce highly excessive amounts of hormone, resulting in such syndromes as Cushing's disease, acromegaly, hyperparathyroidism, hyperadrenalism, and similar endocrinopathies.

2. To maintain in tissue culture and in animals hormone-producing tissues and to ascertain the factors involved in quantitative hormone production in vitro.

3. To assess the capacity of new drugs to alter the course and hormonal activity of hormone-producing tumors.

Methods Employed: Hormone-producing tumors from patients with one of the above-named syndromes are obtained at surgery and carried in tissue culture in irradiated, cortisonized rats and hamsters. The supernatant fluid from the cultures are tested for hormonal activity by appropriate bioassay methods and the quantity recovered compared with the content of a piece of tissue exactly equivalent to that originally placed in culture.

Methods Employed (con't.)

Patients presenting such syndromes are completely characterized endocrinologically and metabolically before and after therapy in order to ascertain the relative effects of various forms of therapy.

Major Findings: 1. Studies on choriocarcinoma and related trophoblastic tumors in women.

(a) Sixty eight women with metastatic choriocarcinoma and related trophoblastic disease have been admitted to the Endocrinology Service during the past five years. Each patient has undergone comprehensive study from both an endocrinological and chemotherapeutic standpoint. The collected histories of these cases provides a massive body of data relating to the natural history of this family of disease processes. The systematic consolidation of these observations on this largest single aggregate of cases of this relatively rare disease correlated with the available histological data will constitute the basis for a comprehensive monograph on this subject.

(b) Review of the chemotherapeutic data reveals that 45% of patients treated according to a specially devised regimen of intensive, intermittent administration of methotrexate show sustained and apparently complete remissions, some of which have now exceeded five years in duration. The remainder show initial partial remission for periods ranging from a few months to as long as three years with ultimate recrudescence of disease. This recrudescent disease has proven consistently refractory to therapy with nitrogen mustard, cytoxan, and actinomycin.

Of 14 patients treated with Vincaleukoblastine following the development of methotrexate resistance, significant response has been seen in 6. Two of the latter have shown sustained and complete remission for 8 and 10 months respectively. Additional data on the local and systemic toxicity of this agent emphasize the major hazard of its use, although no instance of irreversible toxicity has been observed in patients receiving appropriate medical and nursing support.

Preliminary clinical pharmacological study of podophylotoxin, which has been shown to inhibit choriocarcinoma in the hamster, (see Project 808) has been carried out in one female patient who had proven refractory to all other forms of therapy. It was determined that the daily administration of 1.4 mgm. per kilogram intravenously for 5 days is unattended by toxicity of any kind. No significant anti-tumor effect was observed at this innocuous dose. A specially devised preparation for intravenous use has been developed and further studies of this potentially valuable agent are projected.

(c) A review of the endocrinological status of 50 patients with metastatic trophoblastic disease revealed that all of them had normal urinary ketosteroid and corticoid & plasma corticoid levels. All but 2 patients were euthyroid. One patient presented with frank thyreotoxicosis and another exhibited evidence of thyreotoxicosis during the latter stages of her disease. Urinary

Major Findings (con't.)

pregnandiol excretion was within normal range, indicating that the luteal phase of ovarian function was not significantly altered by the high blood levels of chorionic gonadotropin known to exist in these patients. Moreover, four patients in incomplete remission with substantial residual blood levels of chorionic gonadotropin have exhibited repeated, normal menstrual cycles, again reflecting the lack of interference with the normal pituitary-gonadal relationship in the patients.

However, it is known that the human chorionic gonadotropin of pregnancy will prolong the luteal phase of a normal cycle as well as stimulate the interstitial cells of the testis of hypogonadal males. Hence we undertook an assay of the chorionic hormone produced by the tumor patient for the latter effect in 2 hypogonadal boys, in order to ascertain whether this material, like that of normal pregnancy, is gonadotropic for the human. We obtained clear evidence of testicular stimulation in both subjects with a preparation made in our own laboratory from the urine of our tumor patients. Thus, it becomes clear that the refractoriness of the ovaries of the tumor-bearing host to this tumor-hormone involves some special mechanisms for the interference with its peripheral gonadal action. This differs from what is seen during the early phase of the disease when many patients exhibit extreme ovarian enlargement and cyst formation. The subsequent development of refractoriness to an agent now shown to be active in the human requires further study. It is especially significant that such refractoriness is seen in patients whose mammary glands continue to exhibit secretory activity, another endocrinological phenomenon in these patients which requires further analysis.

(d) The potential development of maternal immunization by the invasion of her tissues by a tumor derived from the fetus has been often suggested. However, studies of the red cells and plasma of 35 couples in this regard reflect no such phenomenon. It is also inferred that such immune phenomena do not obtain, since patients in total remission from extensively metastasizing choriocarcinoma can subsequently be impregnated by the same husband. If sustained immune reactions had intervened one would not expect such pregnancies to occur.

(e) Further studies have established that the previously reported failure of the methotrexate-treated patient to convert phenylalanine to tyrosine persists for 4 to 6 weeks after methotrexate intoxication. It is totally unrelated to hepatic or bone marrow toxicity or to therapeutic response, being equally well observed in the methotrexate-resistant patient as in the responsive patient and in the severely toxic as well as in the nontoxic subject. (Dr. Goodfriend).

(f) Extended studies of the distribution of chorionic gonadotropin in the protein fractions of human tumor tissue show that most of the activity, as in the case of serum, is in the beta and gamma globulin fraction. (Dr. Reisfeld and Dr. Nadler).

Major Findings (con't.)

(g) Studies of caloric and nitrogen balance by the classical balance techniques provide data concerning the effects of various anabolic and catabolic hormonal agents in disturbing a previously established equilibrium for calories or for nitrogen or any other element of the diet. Such procedures tell us nothing regarding the adjustments made by patients through altered food intake to such disturbances in metabolic balance. However, we know empirically that patients on protracted intake of a catabolic agent, such as cortisone, frequently stop losing weight either as a result of increased food intake or through some other mechanism which remains to be identified.

We have devised a "paired-tray" procedure for the study of these aspects of the action of various anabolic and catabolic hormones. The patient is permitted to eat ad lib both quantitatively and qualitatively with no restrictions whatsoever and with all his preferences and idiosyncracies catered to. The dietitian prepares two precisely identical trays for each meal or snack. The food from one tray is delivered quantitatively into a homogenizer and ground to a fine puree. The food which the patient leaves uneaten on the other tray is treated identically. Nitrogen, and any other desired chemical elements are determined in both homogenates and the patient's intake is calculated from the difference between what he was offered and what he left. Excretion in stool and urine is followed by the orthodox techniques of collection and analysis.

Four normal adult male volunteers have served as subjects for these studies to date. Each of them has been observed in appropriate control periods and during test periods under the influence of testosterone, triiodothyronine and cortisone. In addition two runs have been made attempting to block the catabolic action of triiodothyronine by massive therapy with the crystalline factors of the B-complex.

It is clear that this technique provides remarkably coherent and reproducible data. The expected anabolic effect of testosterone and the catabolic effect of cortisone and of triiodothyronine have been clearly exhibited. Moreover, food intake data indicate that none of these effects significantly alters food intake over a ten day period. Hence, the initial response to a profound anabolic or catabolic effect is not reflected in a major shift in nitrogen or in a qualitative or quantitative change in caloric intake. Moreover, massive therapy with B-complex factors does not protect against the catabolic effect of triiodothyronine as might be expected from studies by Ershoff in the rat.

Vitamin intake and excretion studies by this procedure reveal that nitrogen retention is quite consistently accompanied by retention of pantothenate and riboflavin and nitrogen loss is associated with increased urinary excretion of these two elements. The latter effect is seen whether the catabolic effect is induced by triiodothyronine or cortisone, thus suggesting a similar mechanism of action for these two widely differing catabolic agents.

These studies establish the paired-tray technique as a useful procedure for the study of what we have termed the "tidal exchange" of nutritional ele-

ments under natural conditions as contrasted with the rigid studies carried out by more classical balance techniques. It is expected that much can be learned concerning the "tidal exchange" in patients with cancer under the influence of various chemotherapeutic agents.

(h) Further studies on the chemotherapeutic potential of o,p'DDD in the therapy of metastatic adrenocortical carcinoma have now included 18 patients. All but 4 have shown a distinct suppression of steroid hormone formation. Seven patients have also shown objective regression of tumor metastases associated with clinical improvement.

The lack of toxicity of this agent for bone marrow, liver and kidney stands in marked contrast to its substantial toxic effects on the gastrointestinal tract and central nervous system. The latter is manifested by nausea, vomiting, diarrhea, and profound anorexia. The neurotoxicity is reflected in somnolence, lethargy, and in a few instances tremor, nystagmus, and ataxia. In each instance these effects have proven reversible on cessation of medication.

Limited studies with a specially devised preparation of o,p'DDD in Lipomul have indicated that little advantage can be obtained through intravenous administration of o,p'DDD.

Adrenal function studies and autopsy data demonstrate that the normal adrenal gland of man can be substantially damaged by o,p'DDD. However, this agent is too toxic to permit its application in lieu of adrenalectomy.

Chemical studies of the blood levels and tissue distribution and metabolism of o,p'DDD have revealed that large but variable proportions of the orally fed drug are unabsorbed and are lost in the stool. There is rapid removal from the blood to body fat depots. After several weeks of therapy the body fat depots, which are meanwhile diminishing because of the patient's anorexia, become saturated and it is possible to then sustain somewhat higher blood levels of drug. This seems to correspond to the most frequent period of steroid and tumor response (Dr. Moy).

(i) Studies of the development of more objective criteria for the determination of the hormonal and metabolic activity of pituitary tumors have been initiated. In relation to acromegaly the development of immunological assays for growth hormone and the use of the chondro-sternal biopsy will be most applicable. Appropriate methodology for the assessment of hormonal profile in patients with chromophobe and other types of pituitary tumor have been devised. A comprehensive study of one patient with a very rare spontaneous ACTH-producing tumor has been completed (Dr. Ross).

(j) A variety of endocrinopathies have been subjected to further endocrinological and genetic analysis. These disorders of somatic development have included gonadal dysgenesis, adrenogenital syndrome, various forms of pseudohermaphrodism, and true hermaphroditism. Tissue culture methods are

Major Findings (con't.)

being developed for the complete cytogenetic profiles presented by these cases. In addition, such functional disorders as adrenal hyperplasia, Stein-Leventhal Syndrome, sexual precocity, hypogonadism, and various patterns of menstrual dysfunction are under study.

(k) In collaboration with Dr. H. Wilson of NIAMD, the following aspects of steroid metabolism in adrenal cancer have been started:

1. Methods have been devised for the isolation and quantitation of some important delta-5-3-beta-hydroxysteroids, delta-5-pregnenetriol, delta-5-pregnenediol, and delta-5-androstenediol.

2. In all patients with functioning adrenal cancer there was an elevated excretion of delta-5-pregnenetriol. The percentage increase in the excretion of this compound compared to the normal was greater than for dehydroepiandrosterone. In one subject, the absolute amounts of delta-5-pregnenetriol excreted considerably exceeded the excretion of dehydroepiandrosterone. In 4 of 5 patients, the excretion of delta-5-pregnenediol and delta-5-androstenediol was increased when compared with the normal subject or with the patient with Cushing's Syndrome.

3. In order to localize sites of relative enzymatic blocks in adrenal cancer, the following urinary metabolites were isolated and measured: androsterone, etiocholanolone, dehydroepiandrosterone, 11-beta-hydroxyetiocholanolone, 11-beta-hydroxy androsterone, 11-ketoetiocholanolone pregnanetriol, tetrahydro compound S, tetrahydrocortisol and tetrahydrocortisone. The observations suggested these conclusions.

11-beta-hydroxylation is generally impeded in adrenal cancer. In two cases, there was a complete block of 11-beta-hydroxylation.

Pregnanetriol is excreted in elevated amounts by many patients with adrenal cancer and this cannot be used to distinguish between cancer and hyperplasia as has been suggested by others. 21-hydroxylation is variably impeded in adrenal cancer.

About 50% of adrenal cancers have a lack of the 3-beta-hydroxy dehydrogenase system thus resulting in synthesis and excretion of the 11-deoxy ketosteroids and the delta-5 3-beta-hydroxymetabolites.

The demolase converting 17-hydroxypregnenolone to dehydroepiandrosterone cannot handle the amounts of precursor presented to it.

4. One successful study was obtained from our attempts to identify the precursor of dehydroepiandrosterone using C14-labelled cholesterol. The data did not support the hypothesis that dehydroepiandrosterone arises from exogenous cholesterol.

The large increases in etiocholanolone excretion have suggested that its

M[illegible] Findings (con't.)

precursors must be other than androstenedione and dehydroepiandrosterone. The use of tritium-labelled dehydroepiandrosterone in 4 patients with adrenal cancer demonstrated that over 60% of the urinary etiocholanolone arose from sources other than dehydroepiandrosterone (Dr. Lipsett).

(l) In collaboration with Dr. L. Garren of the Section of Therapeutics, we examined several aspects of cortisol metabolism in euthyroidal hypermetabolic states. It was shown that cortisol is metabolized at a normal or slightly slowed rate and that cortisol secretion is increased. Thus the effect of hyperthyroidism on cortisol metabolism is probably due to a specific action of thyroxine and not due to hypermetabolism, per se (Dr. Lipsett).

(m) A patient with post-natal adrenal hyperplasia has been studied. The steroid pattern was similar to that of congenital virilizing adrenal hyperplasia. Thus the presumed deficit of 21-hydroxylase may not be apparent for some years (Dr. Lipsett).

Significance to the Program of the Institute: Further understanding of the conditions permitting the excessive hormonal production of tumors of endocrine origin is basic to our understanding of the metabolism of neoplastic tissue generally. In addition, further analysis of abnormalities in somatic growth and development should provide data pertinent to the problems of tissue growth generally.

Proposed Course of Project: Further elaboration of these studies with major emphasis on biochemical and pharmacological control of hormone-producing tumors and anomalous tissue growth is projected.

Part B included.

Attachment I
Serial No. NCI-803(C)
Endocrinology Branch

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Lipsett, M. B. and Riter, B.: Urinary Ketosteroids and Pregnanetriol in Hirsutism. J. Clin. Endocrinol. & Metab. 20: 180-186, 1960.

2. Goodfriend, T. L.: Impaired Conversion of Phenylalanine to Tyrosine After Folic-antagonist Therapy in Women. Federation Proc. 19: 1, (March) 1960.

3. Hertz, R., Lipsett, M. B. and Moy, R. H.: Effect of Vincaleukoblastine on Metastatic Choriocarcinoma and Related Trophoblastic Tumors in Women. Cancer Res. 20: 1050-1053, 1960.

4. Nadler, C. F. and Reisfeld, R.: Gonadotropic Hormone Distribution in Protein Extracts of Trophoblastic Tumors in Man. J. Lab. & Clin. Med. 56: 258-264, 1960.

5. Bergenstal, D. M., Hertz, R., Lipsett, M. B. and Moy, R. H.: Chemotherapy of Adrenocortical Cancer with o,p'DDD. Ann. Int. Med. 53: 672-682, 1960.

6. Lipsett, M. B., Bergenstal, D. M. and Dhyse, F. G.: Metabolic Studies and Human Growth Hormone in Dwarfism and Acromegaly. J. Clin. Endocrinol. & Metab. (to appear).

7. Lipsett, M. B.: A Partial Block in Adrenal Androgen Biosynthesis in Patients with Gonadal Dysgenesis. J. Clin. Invest. (to appear).

8. Hertz, R.: Chemotherapy of Hormone-Producing Tumors of Trophoblastic and Adrenal Origin. Proceedings Canadian Cancer Conference, Fourth Canadian Research Cancer Conference, Honey Harbour, June 1960.

9. Hertz, R.: Therapeutic Trials with Vinca in Patients with Methotrexate. Proceedings of Fourth Canadian Reseach Cancer Conference, Honey Harbour, June 1960.

10. Schmidt, P. and Hertz, R.: Blood Group Factors in Women with Choriocarcinoma as Compared with Those of Their Husbands. Am. J. Obst. & Gynec. (to appear).

11. Hertz, R., Lipsett, M. B. and Moy, R. H.: Endocrinological Evalua-

tion of Patients with Choriocarcinoma and Related Trophoblastic Tumors. Endocrinology (to appear). Presented at meeting of Endocrine Society, Miami, June 1960.

Honors and Awards Relating to this Project:

Dr. Roy Hertz

Invited to participate in meeting of Northern Virginia Medical Societies, April 3, 1960. "Some Instructive Endocrinopathies."

Invited to speak before the Tri-City Obstetrical Society, Philadelphia, Pa., May 3, 1960.

Invited to participate in meetings of XIV National Assembly of Surgeons, Mexico City and Acapulco, Mexico, November 1960.

Participated in Fourth Canadian Cancer Research Conference at Honey Harbour, Canada, June 16, 1960.

Participated in Walter Reed Post-Graduate Course in Gynecology, January, 18, 1960.

Invited to Lecture at Georgetown Medical Service, February and April, 1960.

Invited to participate in Adrenal Cortex Conference held in Glasgow, Scotland, July 13, 1960.

Participated in meetings of the Society of Pelvic Surgeons, October 28, 1960.

Participated in meeting of the New Jersey Obstetrical and Gynecological Travel Club, November 10, 1960.

Invited to speak at annual meeting of the Obstetrics and Gynecologic Department of the Hospital for Women of Maryland, June 1, 1960, in Baltimore, Maryland.

Dr. John Lewis

Invited to speak at Harvard Medical School, December 13, 1960.

Dr. M. B. Lipsett

Appointed Visiting Scientist - Karolinska sjukhuset, Stockholm, Sweden, for 1 year.

Part B (con't.)

Participated in Post-Graduate Course at Providence Hospital, Washington, D. C. 1960.

Invited to lecture at Uppsala Medical School, Sweden.

Invited to lecture at University of Stockholm Medical School, Stockholm, Sweden.

Invited to lecture at University of Gottenburg Medical School, Sweden.

Serial No. NCI-808(C)
1. Endocrinology Branch
2. Endocrinology Service
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Endocrine Factors Governing Hormone-Induced Tissue Growth and Tumor Formation in Animals; Bioassay of Blood and Urine from Cancer Patients.

Principal Investigators: Dr. Roy Hertz, Dr. Griff T. Ross

Other Investigators: Dr. William W. Tullner, Mr. Sidney Siegel, Mr. Philip Rayford, Mr. Howard Erwin, Dr. Saul Genuth, Dr. John Lewis, Dr. Howard Schwartz

Man Years (calendar year 1960):
Total: 14
Professional: 8
Other: 6

Patient Days (calendar year 1960):
Patients
Days

Project Description:

Objectives: 1. To gain basic information concerning endocrinological and nutritional factors governing hormonally conditioned growth and involution in organs such as the uterus, breast and prostate which are the frequent seat of hormone-sensitive neoplasms.

2. To develop and improve bioassay methods for the quantitation of hormones in the blood and urine of patients with cancer who are either receiving various forms of hormonal therapy or who present unusual hormonal patterns.

3. To determine the biological properties of steroid and related compounds in terms of their potential usefulness in cancer therapy.

Methods Employed: Standard methods of biological study are employed. These include:

1. The maintenance of animals of various species on specified hormonal or nutritional regimens.

2. Daily observations of physical and behavioral changes in such animals.

3. Complete autopsy of test animals with detailed weighing and chemical analysis and histochemical study of specific organs.

4. Surgical alteration of the endocrine status of animals by gonadectomy, adrenalectomy, hypophysectomy, thyroidectomy, parabiosis, hysterectomy and other operative procedures.

Major Findings.

1. (a) Further effort has been extended toward the development of additional strains of human choriocarcinoma as heterologous transplants in the untreated hamster. Each available specimen of human choriocarcinoma obtained at surgery or at autopsy has been transplanted to both cortisonized and untreated hamsters. A total of 35 such trials has now yielded 6 well established strains. Each strain has been characterized as to its growth characteristics, hormone production and content and response to several chemotherapeutic agents known to affect trophoblastic tumors in man, namely methotrexate, cytoxan, and vincaleukoblastine. All strains, including those taken from patients exhibiting resistance to all three agents exhibit sensitivity to these same drugs. These findings have considerable pertinence to the problem of the relationship of animal screens to the potential clinical usefulness of available drugs.

(b) A remarkable constancy in gonadotropic hormone content has been noted in original specimens and in the several strains of choriocarcinoma. This indicates that the individual trophoblastic cell is producing hormone at a nearly marginal or optimal amount. Hence the remarkably close correlation of urinary titers with extent of tumor in the patient appears to have a sound biochemical basis.

(c) Among a wide variety of agents tested for possible anti-tumor effect against human choriocarcinoma in the hamster, podophylotoxin, alpha-peltatin and beta-peltatin have been found to have substantial effectiveness. Among these podophylotoxin appears to be the most effective and has the widest margin of safety.

A special preparation of this poorly soluble compound in propylene glycol and Lipomul has been devised. Acute and chronic toxicity studies in the dog indicate its approximate limits of tolerance and provide a basis for initial clinical trial (see project 803).

(d) Studies in the hamster show that complete withdrawal of food, but not water, permit survival for up to 14 days. Such completely starved animals support the growth of implanted choriocarcinoma with no qualitative or quantitative change from that seen in the fed controls. Thus factors of food intake in connection with drug-effects on tumor growth can be considered to be very limited.

(e) Attempts to transfer choriocarcinoma tissue to tissue culture have thus far yielded cultures capable of survival and hormone production of limited duration. After 30 days at most the cultures lose their vitality and hormone production. Methods for the more sustained culture and more accurate quantitation of cell populations are being developed.

Another aspect of the efforts of the tissue-culture laboratory relates to the development of proper cultures for cytogenetic analysis in appropriate problem cases (Dr. Ross).

Major Findings (con't.)

(f) The in-vitro synthesis of gonadotropic hormone by surviving cells has encouraged attempts to prepare cell-free systems from hamster-choriocarcinoma tissue which will synthesize this hormone. A new method for the detection of minute amounts of hormone by titrating its presence against a specific anti-serum in the rat-hyperemia test has been devised. Also cell-free preparations have been successfully prepared by differential centrifugation and the major hormonal activity is shown to remain in the supernatant fluids. With these materials in hand it should now be feasible to further investigate the biosynthetic potential of various cell-free preparations provided with a variety of substrates (Dr. Genuth).

(g) As a part of a comprehensive comparison of the enzyme profile of choriocarcinoma tissue with that of normal human trophoblasts, the steroid dependent transhydrogenase and dehydrogenase content of both types of tissue has been quantitatively compared. It is clear that the tumor tissue contains only a minute fraction of the activity found in normal placenta. This enzymatic loss supports the Greenstein hypothesis concerning the progressive loss of enzymatic specificity in malignant tissue.

In addition histochemical studies on the respective localization of the steroid dependent dehydrogenase and transhydrogenase in human placenta reveals a distinct localization for each, thus supporting the view that these two activities reflect separate enzymes rather than a single enzyme. (Dr. Kellogg).

(h) A highly potent concentrate of chorionic gonadotropin prepared from the urine of tumor patients has been compared with purified hormone prepared from the urine of normal pregnancy. Certain chemical and biological properties permit the inference that the tumor hormone may represent a chemical variant from that found in normal pregnancy. Further analysis of these differences may permit some indication of the nature of the biochemical deviations from normal which characterizes trophoblastic malignancy (Dr. Reisfeld).

(i) The antigenicity of human chorionic gonadotropin from pregnancy and from tumor-sources is demonstrable by both anti-hormone formation and by more classical anti-body formation as demonstrable by precipitation, complement fixation and agglutination inhibition tests. Initial studies directed toward the immunological differentiation between tumor and pregnancy hormone by bio-assay of anti-serum reveal a relative but not absolute specificity of their respective anti-sera. Further elaboration of these quantitative differences should ultimately provide a basis for differential diagnosis of pregnancy from tumor. In addition, preliminary studies of the behavior of these respective anti-sera in Auchterlony plates suggest that some differentiation may also be possible by this technique, as well. (Dr. Schwartz and Dr. Lewis).

(j) In an attempt to evolve more highly sensitive and specific bio-assay methods for gonadotropin we have attempted to measure the hormone-induced increase in vascularity of the ovary of several rodent species as reflected in increased uptake of radio-iodinated serum albumin. Although good

Major Findings (con't.)

quantitation can be carried out by this method, no increased sensitivity over more conventional methods is encountered. Similar results have been obtained in male animals using the testes as an end-point. A survey of the potential usefulness of other species is in progress (Mr. Siegel).

(k) The problem of varying sensitivity of test mice in response to administered gonadotropin is one of great practical concern for the accurate evaluation of chemotherapeutic response in patients with trophoblastic disease. Accordingly, great effort has been exerted toward the elimination of all genetic, nutritional and environmental variables. This has led to the identification of an insecticide (methoxychlor) used on these animals as an inadvertent source of exogenous estrogen. In addition, estrogen contamination of commercial stock rations has also been demonstrated. Notwithstanding extended attempts to eliminate such sources of error, it remains difficult to obtain constant results in such bio-assay systems. This imposes a very serious handicap upon the accurate conduct of our chemotherapeutic studies in man.

(l) The monkey, macacus rhesus, provides a close parallel to the human for the study of reproductive function in the female. A modest breeding program has been developed within the limitations of available facilities. Bio-assay of the urine and serum of bred animals for chorionic gonadotropin has provided a 100% accuracy in the diagnosis of pregnancy in 50 consecutive animals. In addition, about 150 negative tests have been shown to reflect lack of pregnancy. The hormone may be detected from day 18 to 35 of pregnancy but is not detectable both before and after this interval. Correlated studies in 8 monkeys reveals that the hormone is detectable in the trophoblast itself only during this same period. Moreover, hormone levels in uterine vein blood corresponds exactly to that seen in the peripheral blood, indicating a fairly long half-life for this hormone in the circulation.

Chemical concentration of the chorionic hormone from the pooled urine of pregnant monkeys indicates a close similarity between monkey hormone and human hormone. Attempts to differentiate the monkey from the human hormone by immunological means have been recently undertaken.

Further efforts to produce the dissemination of chorionic elements into the peripheral tissues of the monkey in order to simulate human trophoblastic disease have failed. Since fetal death or the termination of pregnancy are so frequently correlated in time with the development of the human disease, we have excised the 25 day old fetus in 4 monkeys without disturbing the blood supply of the associated chorion. In each case this led to the prompt evacuation of the uterus with no persistence of viable chorionic tissue. It is felt, however, that this biological system offers the best available possibility for the experimental reproduction of the human disease pattern and further effort is being directed along these lines.

Proposed Course of Project:

It is expected to continue and extend the foregoing studies along the lines already indicated.

Part B included.

Attachment I
Serial No. NCI-808(C)
Endocrinology Branch

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Kellogg, D.: The 17-beta-estradiol Sensitive Enzymes of Human Choriocarcinoma. Federation Proc. 19: No. 1, March 1960.

2. Hertz, R.: Gonadotrophin and Adrenocorticotropin from Rat Pituitary Homografts as Manifested by Host Response to Chorionic Gonadotrophin and Amphenone. Endocrinology 66: 842-844, 1960.

3. Goodman, A. D.: Studies on the Effect of Omega-methyl-pantothenic Acid on Corticosterone Secretion in the Rat. Endocrinology 66: 420-427, 1960.

4. Kellogg, D. A.: Histochemical Localization of Human Term Placental 17-beta-Oestradiol Dehydrogenases: Implications for the Transhydrogenase Reaction. Nature 187: 4739-4763, 1960.

5. Reisfeld, R. A. and Hertz, R.: Purification of Chorionic Gonadotropin (HCG) from the Urine of Patients with Trophoblastic Tumors. Biochim. et Biophys. Acta 43: 540-543, 1960.

6. Hertz, R.: Suppression of Human Choriocarcinoma Maintained in the Hamster Cheek-pouch by Extracts and Alkaloids of Vinca Rosea. Proc. Soc. Exper. Biol. & Med. Proc. Soc. Exper. Biol. & Med.105: 281-282, 1960.

Honors and Awards relating to this project:

Dr. Roy Hertz

Elected Vice-President of The Endocrine Society, Miami Beach, Florida, June 1960.

Participated in Howard University Physiology Seminar, January 5, 1960.

Acted as advisor to Secretary Fleming and testified at Estrogen Hearings, January 1960.

Chairman, Symposium on Steroids of American Chemical Society, Cleveland, Ohio, April 12, 1960.

Chairman, Section on Thyroid of Federation meetings, April 11, 1960.

Participated in Conference on Ovulation, Harvard University, Boston, Feb. 26-28, 1960.

Serial No. NCI-810
1. Endocrinology Branch
2. Endocrinology Service
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Pharmacological and Physiological Studies on Alteration of Endocrine Function in Animals.

Principal Investigator: Dr. William W. Tullner

Other Investigators: Dr. Roy Hertz, Dr. Griff T. Ross

Man Years (calendar year 1960):
Total: $3\frac{1}{2}$
Professional: $1\frac{1}{4}$
Other: $2\frac{1}{4}$

Project Description:

Objectives: 1. To determine which of the anterior pituitary trophic hormones regulate the biosynthesis of those adrenal steroids (particularly androgens) which subsequently stimulate growth and secretion of the secondary sex accessories associated with carcinoma, such as the male prostate. To identify chemically and biologically the adrenal steroids secreted.

2. To evaluate selected synthetic compounds for their capacity to alter endocrine biosynthetic pathways or to destroy specific hormone-producing cells. To use selective cell destruction as a means of learning more about processes of cell regeneration with respect to morphological and functional differentiation.

3. To investigate the patterns of anterior pituitary hormone secretion in patients with endocrine tumors before and after surgical or chemical alteration of endocrine status.

Methods Employed: Normal animals on standard stock diets were used both for pharmacological studies and surgical procedures.

Hypophysectomized dogs with cannulated lumboadrenal veins were used for patient plasma ACTH assay (Hume and Nelson). Other surgical techniques included adrenalectomy, hypophysectomy, castration, parabiosis and endocrine tissue transplants.

Standard methods were used for bioassay, histology, paper chromatography and chemical determination of steroid hormones.

Major Findings: 1. Studies on the interrelationships of the anterior pituitary, adrenal cortex and the reproductive system.

A. Results of studies on the nature of the anterior pituitary hormonal pattern required to stimulate adrenal androgen secretion show that: in castrate male rats, exogenous adrenocorticotropin (ACTH) produced a significant increase in ventral prostate weight over that of untreated animals, together with transformation of the prostatic epithelium from the atrophic non-secretory to the secretory condition; exogenous prolactin alone did not alter the weight or histology of the castrate male rat ventral prostate, but chronic parenteral administration of the highly purified preparations of prolactin and ACTH promoted greater tissue growth than that induced by ACTH alone. Further, a dose of ACTH was determined which produced adrenal weight increase without increasing ventral prostate weight (sub-threshold adrenal androgen secretion). Prolactin administered concurrently with this level of ACTH produced ventral prostate weight increase of more than 100%. Neither highly purified growth hormone nor luteinizing hormone effected an increase in adrenal or ventral prostate weights when given alone or in combination with ACTH.

B. The action of ACTH or prolactin plus ACTH in stimulating ventral prostate growth did not occur in adrenalectomized, castrate animals at similar dose levels. Thus the observed response is adrenal-dependent.

C. Experiments on the hypophysectomized, castrate male rat revealed that neither exogenous ACTH nor ACTH plus prolactin altered the weight or histology of the ventral prostate although the weight of the adrenals was markedly increased over that of uninjected controls. These results indicate that in addition to the pituitary trophic hormones (ACTH and Prolactin) required for adrenal androgen production, there is a further requirement for unknown pituitary factors in the stimulatory effects on the prostatic tissue. These studies are being continued in order to learn more about the unidentified pituitary synergist(s). It is also planned to determine the adrenal androgens responsible for the observed effects on the ventral prostate by means of paper chromatograms and other chemical methods of identification.

2. Studies on adrenocortical inhibitors.

A. In continuation of studies of the drug o,p'DDD (2,2-bis(2-chlorophenyl-4-chlorophenyl)-1,1-dichloroethane), duration of treatment and drug dose was correlated with degree of cellular destruction in the adult dog. From this data, experimental animals were prepared in which cell damage as determined by histological means was confined to the zona reticularis and zona fasciculata with the zona glomerulosa appearing to be normal. Adrenal biopsies and adrenal vein blood samples were taken on the day following cessation of drug treatment. At this time the adrenal secretion of 17-hydroxycorticosteroids was reduced to extremely low levels (0.5 mcgm/min.). These animals were then sacrificed at intervals from 9 to 30 days. A gradual functional recovery of the adrenal cortical biosynthetic process was observed as determined by measurement of adrenal venous secretion of hydro-

Major Findings (con't.)

cortisone and other 17, 21-dihydroxy-20-ketosteroids which returned to normal (17-hydroxycorticoids 6.0 to 8.0 mcgm/min.)in approximately 4 weeks. Sections of the adrenal cortex taken at autopsy demonstrated a progressive regeneration of the zona fasciculata as the post-treatment interval increased. The newly formed cells of the zona fasciculata were initially few in number and contiguous to the zona glomerulosa cells with the regenerating cells assuming a centripetal direction of development. These experiments demonstrate the development of zona fasciculata cells from those of zona glomerulosa. The reason for resistance of the zona glomerulosa to drug damage is not known. In vitro adrenal slice studies by Giroud and Stachenko (1959) on the functional zonation of the adrenal cortex indicate that the primary site of aldosterone secretion is the zona glomerulosa whereas the zona fasciculata is concerned with synthesis of hydrocortisone and corticosterone. This would indicate a multipotent capacity for the zona glomerulosa cell which can give rise not only to enzyme systems required for aldosterone production but also to cells capable of producing 17-alpha-hydroxylating systems. These experiments are being continued. The principal current problem is to determine the pattern of steroid hormones secreted at the time of maximum adrenocortical damage together with changes found during the regenerative process.

B. Endocrine studies of the insecticide, Methoxychlor (a mixture of 90% 1,1,1-trichloro-2,2-bis(p-methoxyphenyl) ethane and 10% other isomers of Methoxychlor).

Bioassay problems arising from uterine stimulation in untreated immature female mice led to the finding that an insecticide used for ectoparasite control by the Animal Production Section reproduced the observed uterine weight increase. The fact that this insecticide, Methoxychlor is an analogue of DDD and other adrenocortical inhibitors which we have been investigating, led to further characterization of its effects on the endocrine and related systems.

Methoxychlor stimulated uterine weight increase in the ovariectomized, immature female mouse at total doses of 0.5 mg given orally for 3 days, with maximum stimulation at a 5.0 mg total dose (LD50 7.5 mg). Similar uterine growth effects were noted in the ovariectomized female rat at higher doses. The treated mouse presented an atypical vaginal response. The vaginal smear contained an abundance of leucocytes only. However, vaginal cornification, characteristic of the estrogens, was observed in the vaginal smear of the Methoxychlor-treated rat. Adrenalectomy in the ovariectomized rat or mouse did not alter the uterotrophic action thus excluding the possibility that derangement of adrenocortical biosynthetic enzyme systems by Methoxychlor might lead to excess estrogen production by the adrenal cortex. Acute oral administration of Methoxychlor to female hypophysectomized rats resulted in uterine enlargement demonstrating that the uterine action of the compound is not mediated by the anterior pituitary. Intact female-castrate female pairs of parabiotic rats were used to determine the pituitary depressant action of the insecticide. Methoxychlor, in common with the estrogens, inhibited the gonad stimulating effect of the anterior pituitary gonadotrophins.

Major Findings (con't)

In male rats, pair fed for 3 weeks, with half the group receiving 1% Methoxychlor in the diet, the mean weight of the testes from untreated animals was seven times that of the treated rats and the ventral prostate weights of the latter were only 20% of those from pair fed controls. Methoxychlor, at this level, produced Leydig cell atrophy and a failure of spermatogenesis. They kidneys of treated rats were cystic and larger than normal with markedly dilated tubules.

Adult mongrel dogs were given Methoxychlor (200 mg/kg) by mouth for 15 days with no alteration in adrenocortical 17-hydroxycorticoid secretion. The histology of the adrenal cortex in these dogs was also normal.

The highly purified isomer p,p'Methoxychlor exerts a weak uterotrophic action in the rat and mouse at maximum dose levels indicating that the effects noted are due in part to still unidentified isomer(s) in the technical material. Further studies on the endocrine action of Methoxychlor in the male rat are in progress. Other isomers than the p,p' compound will be tested when available in order to identify the active uterotrophic agent.

C. Since the adrenal has been implicated as a mediator of progestational action, compounds such as desoxycorticosterone and 9-alpha-chloro-hydrocortisone were assayed in the adrenalectomized-ovariectomized rabbit with the finding that these compounds still elicited their progestational effect. This indicates that adrenal progestins are not responsible for the endometrial changes produced by these steroids.

Several selected diaminodiphenyl compounds have been tested for potency as adrenocortical inhibitors in the dog. Most of these compounds were inactive. Intravenously administered difluorodinitro DDD was found to be as active as o,p' DDD in blocking synthesis of corticosteroids but appeared to have no advantages over the latter compound.

D. Studies have been continued on the role of the adrenal cortex as a source of progestins. The progestational response to amphenone and related compounds by the immature rabbit uterus (Hertz et al.) was shown to be a result of adrenal progestin secretion. Adrenal vein blood extracts from methylenedianiline treated rabbits was run in several paper chromatographic systems. This analysis has demonstrated the presence of a compound more polar than progesterone which had a progestational action when eluted and reinjected into estrogen-primed immature female rabbits. Further work on the identification of this compound is underway following collection and extraction of large volumes of adrenal vein blood from treated and untreated rabbits.

3. Collaborative investigation characterizing anterior pituitary hormone secretion in clinical endocrinopathy.

Circulating levels of anterior hormones, such as ACTH, have been measured in the plasma of patients with various endocrinopathies before and after

Major Findings (con't.)

treatment or surgery. ACTH content of plasma is measured by the response in adrenal corticosteroid output in the hypophysectomized dog using standard doses of ACTH as reference. Nelson et al. (1960) have reported ACTH-producing chromophobe adenomas of the pituitary following bilateral adrenalectomy for Cushing's Syndrome. Furth has also reported ACTH-producing pituitary tumors in mice following irradiation. The findings in this investigation do not permit conclusions at this time.

Significance to Bio-medical Research and the Program of the Institute.

A knowledge of the factors which promote or inhibit endocrine tissue growth and/or secretion, provides fundamental information needed to understand the regulation and control of abnormal tissue growth.

Proposed Course of Project.

It is expected that these studies will be continued as indicated under the individual reports on major findings. collaborative clinical study on circulating anterior pituitary hormones in various endocrinopathies is being expanded with the development and standardization of quantitative methods for assay of specific hormones. Chemical inhibitors of hormone secretion will be investigated as these become available.

Part B included.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

1. Tullner, William W. and Hertz, Roy. Effect of Intravenous o,p' DDD on plasma 17-hydroxycorticoid Secretion in the Dog. Endocrinology 66: 494-496, 1960.

2. Tullner, William W. Endocrine Effects of Methylenedianiline in the Rat, Rabbit and Dog. Endocrinology 66: 470-474, 1960.

3. Tullner, William W. Uterotrophic Action of the Insecticide Methoxychlor. Science (in press).

Honors and Awards relating to this project:

September 1960 - appointed Lecturer in Chemical Endocrinology, Chemistry Department, Georgetown University Graduate School, Washington, D. C.

Serial No. NCI-814
1. Endocrinology Branch
2. Endocrinology Service
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: The Preparation and Evaluation of Biotin Antagonists in the Study of Biotin Metabolism as an Approach to Cancer.

Principal Investigator: Mr. Frederick G. Dhyse

Other Investigators: Dr. Roy Hertz

Man Years (calendar year 1960):
Total: ¼
Professional: ¼

Project Description:

Objectives: The principal objectives of this project are to develop and prepare biotin antagonists that are particularly effective in producing deficiencies of this vitamin in laboratory animals as well as in microbial systems. A possible route of application of such deficiencies to cancer studies is described under "Significance to the Program of the Institute." Furthermore, appropriate antagonists are to be used to "block" certain biochemical reactions involved in the study of Biotin Metabolism in bacteria.

Methods Employed: Biotin antagonists are prepared by organic chemical synthesis or isolated from natural sources, and evaluated by microbiological assay and biological testing in rats and mice. Paper chromatography is extensively used in the separation and identification procedures.

Major Findings: The findings (described in last year's report) that excess biotin added to cultures of L. arabinosus gave rise to a 5-fold increase in secretion of "folic acid" by this organism was investigated further. The "folic acid" secreted into the medium was found to be pteroyl tri-glutamic acid (PTGA), rather than pteroyl-glutamic acid (Folic acid or PGA). Evidence from paper chromatography (bioautography) indicated the "folic acid" inside the cells (after enzyme digestion) to be in the form of PTGA also. These naturally occurring substances were compared chromatographically with a synthetic PTGA ("Terapterin" from Lederle Labs.) and found to have comparable Rf values.

Omission of p-aminobenzoic acid from the medium in which L. arabinosus is grown does not reduce the growth of this organism as measured by turbidity and lactic acid production. However, the "folic" content of these cells was reduced to about 1/10 normal values (L. casei folic assay). This observation is in line with the known role of paba as a precursor of folic acid.

Major Findings (con't.)

In contrast to the findings with L. arabinosus, two different strains of E. coli did not show any secretion of "folic" acid into the medium, although the E. coli cells themselves were rich in folic activity (L. casei assay organism). In fact E. coli supernatant was actually inhibitory to the growth of L. casei under conditions of the assay.

Significance to the Program of the Institute:

Evidence from the literature indicates biotin may be required for the tissue synthesis of folic acid, and findings presented in this report show a positive effect of biotin on the synthesis of folic acid in micro-organism. In view of the relative success of folic antagonists in the treatment of cancer, and because of the drug resistance later developed to such treatment, an appropriate inhibitor of biotin's role in folic acid synthesis might make present treatment with anti-folics more effective.

Proposed Course of Project:

1. Germ-free animal studies will be pursued in order to verify the as yet unconfirmed observation of T. D. Luckey that a large increase in folic acid excretion took place when biotin was given to biotin-folic deficient germ-free rats.

2. Further study of the relationship of biotin to folic acid, in micro-organisms, directed toward a more precise evaluation of the nature of this relation. Development of antagonists to biotin and to intermediates in this biotin-folic function.

3. Evaluation of biotin antagonists, and also antimetabolites of certain biotin vitamers in microbiological systems where drug resistance has been developed to anti-folics. Extension of such studies to rats and hamsters with tumor resistant to methotrexate.

4. Separation and isolation of the biotin vitamers described in a publication of previous findings in connection with this project.

Part B not included.

Serial No. NCI-4-709 (c)
1. General Medicine Branch
2. Chemotherapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.:

Project Title: The Chemotherapy of Acute Leukemia

Principal Investigators: Emil Frei III and Emil J Freireich

Other Investigators: L. Schroeder and M. Karon

Cooperating Units: Clinical Pharmacology and Experimental Therapeutics Service
Jefferson Medical College
Walter Reed Army Medical Institute
Jackson Memorial Hospital
Medical College of Virginia
Roswell Park Memorial Hospital
Emory University
Bowman-Gray School of Medicine
Mount Sinai Hospital, New York
Philadelphia Children's Hospital
Mayo Clinic
Dartmouth University Hospital

Man Years (calendar year 1960) Patient Days (calendar year 1960)
Total: 7
Professional: 5
Other: 2

Project Description:
Objectives: 1. To study quantitatively the maximum usefulness of drugs that are known to have activity against acute leukemia.
2. To discover and test in humans new chemical compounds which can alter the course of acute leukemia.
3. To study the effects of these compounds on the host.
4. To test the usefulness of animals and other screening techniques by the collection of quantitative clinical data in man.
5. To integrate studies of the mechanisms of action and resistance and pharmacology with clinical cancer chemotherapy.

Part B included YES

Methods Employed:

1. Case Material: All patients referred to the Clinical Center with the diagnosis of acute leukemia are admitted to the study. In addition there are 12 hospital units other than the NCI participating in most of the below mentioned therapeutic trials.

2. Criteria: Objective criteria for the evaluation of disease activity have been developed and applied. Their applicability and meaningfullness is under continuing scrutiny.

3. Pharmacology: In cooperation with the Laboratory of Clinical Pharmacology, pharmacologic disciplines are applied to the drugs tested. These studies are divided into three major categories:

a) Toxicity studies of compound of potential antitumor activity given to humans for the first time.

b) Absorption, distribution and excretion studies of known and potential antitumor compounds.

c) Analysis of the influence of varying dosage schedules and routes of administration of antitumor activity and toxicity.

4. Experimental Design: In cooperation with biostatisticians and the Clinical Pharmacology Section therapeutic trials were designed. These include:

a) Cooperative comparative therapeutic trial designed to obtain among other things, quantitative information of the relative effects of antimetabolites of proved antitumor activity in man.

b) Sequential Analysis - New drugs are tried in patients with acute leukemia who have already received conventional antileukemic therapy. If the pilot studies suggest possible human antileukemic activity further studies are designed. Initially these have been performed by administration of the drug to be tested to all patients qualifying.

5. Improved methods have been developed for the separation of white cells from the peripheral blood. Using these techniques, studies of drug action and drug resistance at a cellular level, using biochemical techniques that have been employed in similar studies on mouse ascites tumors, are underway.

Major Findings:

1. Protocol II - This study has been completed and is being prepared for publication. The results are essentially the same as those given in the 1959 Project Report.

2. Protocol III - In this study all children with acute leukemia are initially treated with prednisone. When remission occurs the patients are placed on either 6-MP or placebo using the double blind random allocatinn technique. Remission duration was used as the parameter of

response. 102 patients have been entered into the study (18 by NCI). This study has been completed and reveals the following:

a) 6-MP antileukemic activity was demonstrated by the utilization of only a small number of patients.

b) Overall survival for placebo patients was not impaired (because 6-MP could be subsequently used to induce remission in these patients).

Thus this novel experimental design has important advantages in evaluating new agents as follows:

a) The parameter of response is quantitative rather than qualitative which means that differences can be detected with much smaller numbers of patients.

b) New agents can be studied early in the course of the disease when more patients are available.

c) Toxicity of new agents can be better evaluated as patients in remission are not beset by the frequent complications of active leukemia.

3. Dichloromethotrexate (DCM). This study has revealed the following:

(a) DCM is not more active than MTX when given orally to patients with acute leukemia.

(b) DCM given subcutaneously is currently under study. (20 patients to date). Preliminary evaluation would indicate that even parenteral DCM is not more active than MTX.

4. Cytoxan

110 patients with acute leukemia have been treated with Cytoxan (33 at NCI). The remission rate for children is 16% and for adults 14%. It is concluded that Cytoxan has definite but limited clinical antileukemic activity.

Cytoxan is currently being evaluated using the Protocol III (remission maintenance) design.

5. Methyl-glyoxal-bis-guanylhydrazone. Studies with this compound have revealed the following:

a) It is not absorbed from the human GI tract. This is in contrast to findings in animals.

b) It produces hypoglycemia in a small proportion of patients.

c) In the 8 patients treated at NCI antileukemic effects have been observed in 3 patients and one adult has entered a complete remission.

6. Azauridine - 12 patients with acute leukemia have been treated. A fall in the WBC and decreased organ size has been noted in most of the patients but remissions have not occurred. However, limitations of drug supply have precluded adequate trial in many of these patients.

In chronic myelocytic leukemia azauridine is active in that the WBC and spleen may return to normal and the hemoglobin and platelet values may return to normal.

Azauridine inhibits orotidylic decarboxylase, an enzyme essential to pyrimidine biosynthesis. This enzyme is being measured in human WBC. The following has been observed:

a) The enzyme is profoundly inhibited both in vivo and in vitro by azauridine.

b) The degree of inhibition correlates well with the observed antileukemic effect.

c) Resistance to azauridine (rise in WBC in spite of continued drug administration) correlates well with decreased inhibition of orotidylic decarboxylase. Whether this results from a quantitative enzyme increase or a qualitative change is under study.

7. Nitrosoguanidine. This compound is active against a variety of transplanted rodent tumors and has been studied in 9 patients with acute leukemia. Its mechanism of action is unknown. No antileukemic effect in man has, as yet, been demonstrated.

8. Vincaleukoblastine (VLB). This plant alkaloid is important because of its novel structure, its activity against transplanted animal tumors and particularly its activity against human choriocarcinoma and Hodgkins disease. 16 patients with acute leukemia have been treated with VLB. Though no remissions have been produced the drug is capable of markedly reducing leukemic infiltrations in the spleen and other sites.

9. Imidazolyl thioguanine (ITG). This purine analog has a mechanism of action that is qualitatively similar to 6-mercaptopurine but is quantitatively superior (in terms of therapeutic index) to 6-mercaptopurine in several transplanted mouse tumors. 54 patients with acute leukemia have received the drug and the remission rate is significantly less than that for 6-mercaptopurine.

10. Actinomycin D. This antibiotic is active against certain human tumors (Wilms tumor and embryonal rhabdomyosarcoma). 4 patients have been treated without response.

11. Sarcolysin (l-phenylalanine mustard). This alkylating agent has a better therapeutic index than nitrogen mustard in a variety of quantitative animal tumor screens. In addition there is evidence that its spectrum of human antitumor activity may differ from that of the conventional alkylating agents. 9 patients with acute leukemia have received sarcolysin. Improvement, short of remission, occurred in 3 patients and further studies are planned.

Proposed Course of Project:

1. Studies of conventional agents (6-MP and MTX) will continue. Emphasis in the next 1 - 2 years will be on the study of varioud dose schedules and more particularly the intrathecal administration of these agents in an effort to prevent relapse. All of these studies will require close collaboration with Clinical Pharmacology.

2. Studies of congeners of 6-MP and MTX (e.g. dichloro MTX) will be pursued. Substrate analogs for enzymes in the citrovorum factor biosynthetic path way beyond the site of block of MTX are currently being studied in animals. 6-MP riboside, 9-ethyl-6-thiopurine and 1-methyl 6 thiopurine are being considered for clinical trial because of superior antitumor performance in animals or because of biochemical considerations.

3. Experimental design in the study of new agents will continue to receive major emphasis.

4. The selection of new agents for study is perhaps the most difficult problem. It will be pursued along the following lines:

a) A sufficient amount of quantitative comparative data on drugs in man must be obtained to determine the predictability of the quantitative animal tumor screens currently in use. A reliable screen would, of course, markedly improve the selection of agents for clinical trial. Because of the many variables an attempt will be made to program these data for a computer (A. Pratt, C. G. Zubrod).

b) Expansion of the animal screen to viral and carcinogen induced and spontaneous tumors.

c) Synthesis and study of substrate analogs related to certain biosynthetic pathways in which the NCI has a basic interest (e.g. folic acid).

5. More intensive study of the problem of resistance. The biochemical mechanism of resistance to 6-MP and MTX has been fairly well defined for rodent tumors. Related studies in man are underway which will determine whether similar mechanisms occur and which may, hopefully, suggest approaches to the prevention of resistance.

PHS-NIH
Individual Project Report
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Part B.: Publications

1. Freireich, E. J., Frei III, E., Holland, J. F., Pinkel, D., Selawry, O., Rothberg, H., Haurani, F., Taylor, R., and Gehan, E. Evaluation of a New Chemotherapeutic Agent in Patients with "advanced refractory" acute leukemia. Studies of 6 azauracil. Blood 16:1268, 1960

2. Selawry, O., Frei III, E., Freireich, E. J., Schroeder, L., Gehan, E. A. et al. Effect of Imidazolyl thioguanine on acute leukemia in man. Cancer Chemotherapy Reports, 8:56, 1960.

3. Hoogstratten, B., Schroeder, L., Freireich, E. J., Frei III, E. et al. Cyclophosphoramide in acute leukemia, Preliminary Report. Cancer Chemotherapy Reports, 8:116, 1960.

4. Freireich, E. J. Investigations in Acute Leukemia. NCI Monograph #3, Conference on experimental clinical cancer chemotherapy. Page 149, 1960.

5. Frei III, E. Criteria for Response to Therapy of Acute Leukemia. NCI Monograph #3, Conference on experimental cancer chemotherapy 1960.

Serial No. NCI-4-715 (c)
1. General Medicine Branch
2. Chemotherapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: The Mechanism and Management of the Hemorrhagic Disorder Associated with Acute Leukemia

Principal Investigator: Emil J Freireich, M.D.

Other Investigators: L. R. Schroeder, M.D. and Lawrence Gaydos, M.D.

Cooperating Units: Blood Bank, D.B.S., Dr. Allan Kliman

Man Years (calendar year 1960) Patient Days (calendar year 1960)
Total: 2
Professional: 1
Other: 1

Project Description:

Objectives: a) To identify factors responsible for precipitating gross hemorrhage in thrombopenic acute leukemia patients.
b) To study the role of the platelet in blood coagulation, and hemostasis.
c) To improve the therapy of thrombopenic hemorrhage.

Methods Employed:
a) In vitro and in vivo blood coagulation studies.
b) Column chromatography using cation exchange resins.
c) Analysis of clinical, laboratory and pathological data on patients with hemorrhage.
d) Transfusion of platelets repeatedly to patients to observe effects on hemorrhage and on platelet levels in blood.

Major Findings:

1. Studies of Thrombin - Batch chromatographic techniques have been developed to allow for purification of quantities of thrombin as large as 100 mgm. This material is homogeneous to ultracentrifugal analysis. The instability of thrombin at low ionic strengths at neutral pH has precluded further physical studies such as electrophoresis and diffusion.

2. Quantitative relationship between platelet level and risk of hemorrhage. A retrospective chart review for 92 patients with acute leukemia has indicated a direct relationship between degree of thrombocytopenia and the risk of hemorrhage (expressed as a fraction of the total number of days at such platelet levels).

Part B INCLUDED NO

3. Platelet transfusion studies - utilizing the techniques of plasmaphoresis to supply platelets over 200 units (1 unit = platelets contained in 500 cc of whole blood) have been administered to 20 patients with acute leukemia. Only a single patient was found to be totally resistant to treatment. The remaining 19 all showed some degree of response, allowing for repeated treatments (up to 19 in one patient) covering a span of time up to 100 days. No clear instance of immunization to a single donor's platelets has been seen. All patients showed good clinical control of hemorrhage, correlating well with the measured platelet counts.

Proposed Course:

1. Biochemical studies of thrombin structure will be pursued.

2. Platelet transfusion studies will be expanded to include the study of multiple separate donations of platelets (as with red cells) and to include donor volunteers to expand the plasmaphoresis supply of platelets.

Serial No. NCI-4-719 (c)
1. General Medicine Branch
2. Chemotherapy Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: A Study of the Clinical Manifestation of Acute Leukemia.

Principal Investigators: Emil J. Freireich, M. D. and Emil Frei III, M.D

Other Investigators: L.R. Schroeder, M. D. and Myron Karon, M. D.

Cooperating Units: None

Man Year (Calendar Year 1959): Patient Days (Calendar Year 1959):
Total: 2
Professional: 2
Other: 0

Project Description:

Objectives: To delineate etiologic and pathogenetic factors of the various manifestations of acute leukemia.

Methods Employed: The comprehensive application and correlation of clinical, hematological, radiologic, chemical, microbiological, and histologic methods to the above manifestation in patients with acute leukemia.

Major findings:

1. Meningeal and central nervous system involvement. The nature of the changes in spinal fluid proteins has been studied by paper strip electrophoresis. This studied suggest that the high protein levels are a result primarily of albumen elevation with depression of the pre-albumin (X) component. Studies of intrathecal 6MP-riboside have been initiated in one patient with meningeal leukemia. A dose of 3 mgm/kg has been given without toxicity.

2. A retrospective study of the usefulness of changes in circulating blasts for predicting response to a chemotherapeutic agent has been undertaken. The disappearance of blasts from the circulating blood does separate those who eventually respond to therapy from those who fail to respond. However this parameter does not distinguish

Part B included YES

between inactive compounds and active compounds in patients who fail to respond to Rx.

3. Kidney. A clinical and pathological study of the kidney in 57 patients with acute leukemia has been completed and is being prepared for publication. Perhaps the most significant finding was that 1/3 of the patients had marked renal enlargement in the absence of leukemic infiltration. These patients also tended to have liver enlargement not due to leukemic infiltration. As yet no clinical factors correlate well with this observation. More detailed studies concerning cell size, etc. are contemplated.

4. Nucleic acid - As per previous reports the Chemotherapy Service is collaborating with the Laboratory of Physiology in a study of nucleic acid metabolites in the urine of patients with acute leukemia.

5. Serum proteins - Electrophoretic evaluation of serum proteins in patients with acute leukemia revealed the following: (a) the gamma globulin faction is high in acute myelogenous leukemia (AML) and low in acute lymphocytic leukemia (ALL). (b) Alpha-2 globulin is increased in ALL and (c) the serum protein abnormalities revert toward normal during remission.

Significance to Cancer Research: The continuing scrutiny and definition of clinical manifestations of acute leukemia is of significance to cancer research from many points of view. This is one of the major areas wherein leads to pathogenetic mechanisms may be uncovered. In addition, precise observations are essential to defining the natural history of the disease and this latter is of critical importance to drug evaluation, the major effort of our section.

Proposed Course of this Project: Studies of meningeal leukemia will be extended to further studies of intrathecal 6-MP-R_1 and intrathecal antifolics in large doses with bone marrow protection with intravorum factor.

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Calendar Year 1960

Part B.

1. Shaw, R. K., Moore, E. W., Freireich, E. J., and Thomas, L. B. Memingeal leukemia: Neurol. 10:823, 1960

2. Fahey, J. L. and Boogs, D. R. Serum protein changes in Malignant Diseases The Acute Leukemias. Blood 16:1479, 1960

Serial No. NCI-4-734 (c)
1. General Medicine Branch
2. Chemotherapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Assay of Erythropoietin in bone marrow suspensions

Principal Investigators; Dr. L. R. Schroeder and C. W. Gurney

Cooperating Units: Argonne Cancer Research Hospital and Department of Medicine, The University of Chicago Clinics, Chicago, Illinois

Man Years 0 Patient Days 0

Project Description:

Objectives: To perfect a simple in vitro assay method for erythropoietin.

Methods:) The project was
Patient Material:) not actively
Major Findings:) investigated during
Significance to the Program:) calendar 1960

Proposed Course of the Project:

It is proposed to suspend the project until such time as other work in progress can be completed.

Part B NOT INCLUDED

Serial No. NCI-4-735 (c)
1. General Medicine Branch
2. Chemotherapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: The Effect of Therapy on the Synthesis of Desoxyribonucleic Acid by Leukemic Cells

Principal Investigators: Dr. George Brecher and Dr. L. R. Schroeder

Other Investigators: None

Cooperating Units: Hematology Service, Dept. of Clinical Pathology, Clinical Center

Man Years (Calendar Year 1960)
Total: 3/4
Professional: 1/2
Other: 1/4

Project Description:

Objectives: To study the rate of DNA synthesis of leukemic cells of the peripheral blood and bone marrow in an in vitro system.

Methods: Incubation of leukemic cells with tritium-labelled thymidine and identification of labelled cells by radioautography was used as an indicator of DNA synthesis.

Patient Material: Multiple peripheral blood and bone marrow specimens were obtained from patients with acute leukemia being cared for by the Chemotherapy Service of NCI.

Major Findings: A considerable number of patients with acute leukemia were studied prior to the institution of therapy and 48 hours afterward, and radioautographs prepared for differential counting. The latter is under way but incomplete. In all cases, the degree of labelling prior to therapy was low. Where change was evident after therapy it was in the direction of decreased labelling. Often, only 1/2 - 1% of cells were labelled, necessitating the counting of very large numbers.

Part B NOT INCLUDED

Significance to Cancer Research:

If the effect of therapy on the rate of DNA synthesis by leukemic cells is found to predict eventual responsiveness of the individual's disease, it could result in a tremendous saving in time and patient morbidity.

Proposed course of the project:

Preparations so far completed will be evaluated and the results compared to the effect of the therapy on the patient's disease. If there is an evident correlation between in vitro effect and the patient's response, an attempt will be made to study all new patients. Otherwise, the series will probably be enlarged somewhat before terminating the study.

Serial No. NCI-4-736 (c)
1. General Medicine Branch
2. Chemotherapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: The Demonstration of a Hormone Governing the Production of Platelets

Principal Investigators: Dr. L. R. Schroeder and Dr. Glenn R. Lee

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960) Patient days (Calendar year 1960)
Total: 1 1/2
Professional: 1
Other: 1/2

Project Description:

Objectives: To demonstrate the existence in man and animals of a hormonal substance which governs the production of platelets by the bone marrow.

Methods: Thrombopenia was produced in experimental animals by injections of saponin, by the passage of blood over columns of Dowex-50 resin beads, by prolonged thrombopheresis through an indwelling vascular catheter, and by the administration of specific platelet antibodies.

Major Findings:

At the time of the last report, megakaryocytic hyperplasia had been found in the marrow of one rabbit treated with thrombopenic plasma. Two unsuccessful attempts were made to reproduce this result. Thereafter a much larger group of normal rabbit bone marrows were examined, sampling from multiple sites in each animal. The results revealed a much wider range of megakaryocytic counts in the normal than had been apparent from the earlier smaller sample.

The next group of experiments were performed using rats as recipients of rabbit plasma. This was prompted by a verbal report in March that deproteinized human plasma caused a reproducible thrombocytosis in rats. The administration by a variety of routes of large amounts of plasma from thrombopenia rabbits and from selected human subjects was studied. Neither in the unaltered recipient animal nor

the animal at varying intervals after splenectomy was any response seen. Longer term experiments using plasma ultrafiltrates were equally unrewarding. An antiserum to rat platelets was then produced in the rabbit and used to produce thrombopenia in rats. Plasma harvested from these was then given to normal rats. No alteration in platelet counts was seen. Bone marrow megakaryocytic counts are now under way on these animals.

Significance to the Institute:

Proof of the existence of a hormone governing platelet production will add substantially to an understanding of the homeostatic mechanisms that control bone marrow function in the normal. Derangement of these mechanisms may have a role in the thrombopenia that is a part of the acute leukemias and the thrombocytosis that accompanies chronic myelocytic leukemia.

Proposed course:

Proposed further studies include a week-long continuous infusion of "active" rabbit plasma into normal recipient rabbits, study of the response of splenectomized rats to thrombopenic rats plasma, the effect of reticuloendothelial blockade on platelet production and on the response to infused plasma.

Serial No. NCI 4-740 (c)
1. General Medicine Branch
2. Chemotherapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.:

Project Title: Hypothermia in the chemotherapy of Leukemia - Pilot Study

Principal Investigator: Myron Karon, M.D.

Other Investigators: None

Cooperating Units: Clinical Pathology and Anesthesiology

Man Years (calendar year 1960) Patient Days (calendar year 1960)
Not Applicable

Project Description:

Objectives: 1. To study the effect of the reduction of total metabolism induced by hypothermia on leukemia. 2. To explore the effect of combinations of chemotherapy and hypothermia.

Methods Employed: 1. Animal experiments. Mice with L 1210 will be subjected to hypothermia alone and combinations of hypothermia and chemotherapy and the effects studied with standard methods. 2. Human experiments. Children with acute leukemia will be subjected to prolonged hypothermia. The usual parameters of their disease will be followed.

Major Findings: Studies have not commenced.

Significance to Cancer Research: This study could have broad implications regarding the effect of lowering total body metabolism in neoplasia.

Proposed Course of Project: Should this pilot approach prove feasible and fruitful in the human and concurrent studies in L 1210 prove promising, a large scale project will be undertaken in humans with leukemia and other malignancies.

PART B INCLUDED NO

Serial No. NCI-5-712 (c)
1. General Medicine Branch
2. Solid Tumor Service, Chemotherapy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

A. Comparative Trials of Chemotherapeutic Agents in Patients with Solid Tumors.

B. Initial Therapeutic and Pharmacological Studies of Chemotherapeutic Agents in Patients with Solit Tumors.

Principal Investigators: Emil Frei, III, Clyde O. Brindley, Paul Carbone.

Other Investigators: Myron Karon, Vincent Bono, Robert Scoggins, Harold Fallon, Richard Mechanic.

Cooperating Units: 1) Clinical Pharmacology and Experimental Therapeutics Service. 2) The Eastern Solid Tumor Chemotherapy Group. 3) Radiation Branch, N.C.I.

Man Years (Calendar year 1960)
Total: 4 1/2 years
Professional: 2 1/2
Other: 2 years

Patient Days (Calendar year 1960):

Project Title:

A. Comparative Trials of Chemotherapeutic Agents in Patients with Solid Tumors.

Project Description:

Objectives: To take part in the Eastern Solid Tumor Chemotherapy Group in the application of the principles of the Comparative Clinical Trial in the evaluation of chemical agents in the treatment of human cancer. The present primary objectives of the group are the improvement in the methodology of the Comparative Clinical Trial in human cancer patients and the use of the comparative trial to establish the relative effectiveness of selected chemotherapeutic agents in man.

Part B included YES

These data can be used to (1) evaluate the correlation between the activity of these agents in animal tumor screening systems and in patients with cancer and (2) to establish their relative usefulness in current therapy of human cancer.

Methods Employed:

Several newer methods are being tried in the comparative clinical trials. These include a comparasion of two procedures for final case evaluation. One is by independent vote of the participants in the study after reviewing the case with the type of therapy withheld, the other is a calculation of the degree and duration of response by the statistical office and evaluation of toxicity and overall acceptability of the case by several investigators. Also the usefulness in chemotherapy trials of patients whose tumor masses are not optimum as to measurability are being studied. These include cases with palpable abdominal and pelvic masses and lesions observable through the proctoscope. The principle of sequential analysis is being used, for example for those tumor types which in general have been insensitive to alkylating agents, their inclusion in comparative trials with this type drug has been done by limiting the number of cases entered if the response is consistently negative. The already established principles of the comparative clinical trial of random allocation of cases to various regimines, following of tumor response and toxic side effects of the drug by standardized serial observations and final evaluation of response in a "blind" fashion are adhered to.

Major Findings:

Studies completed in the past year.

1) Thiotepa (TSPA)-Nitrogen mustard (HN_2) study of Melanoma and Hodgkin's disease.

2) X-ray-Nitrogen mustard (HN_2) study of Bronchogenic carcinoma.

3) Oral Dichloromethotrexate (DCM) study in Lymphomas.

1) TSPA-HN_2 study in Melanoma and Hodgkin's disease: In Melanoma the response rate was low (HN_2-4% and TSPA-13%) with both drugs. When the response rate is low differences between drugs can hardly be demonstrated, so it is concluded that Melanoma is not a suitable tumor in which to look for differences between alkylating agents. In Hodgkin's disease there was a definite dose-response relationship, the response rate being much better as the dose is increased. This is to be expected since the alkylating agents are not specific for tumors but are general nucleic acid inactivators;

the maximum effect on the tumor occurs at that dose which is the maximum tolerated by normal cells. At the maximum tolerated doses, HN_2 and TSPA have comparable antitumor effects. With TSPA the lack of local vein irritation and the lack of the very unpleasant gastrointestinal side effects seen with HN_2 would seem to make TSPA the more practical agent.

2) X-ray-Nitrogen mustard (HN_2) study in Bronchogenic carcinoma: In this study the use of X-ray alone is compared to X-ray plus HN_2. Preliminary analysis has indicated that the tumor response or survival time are not improved by the addition of the drug to irradiation therapy alone.

3) Oral Dichlormethotrexate (DCM)-Methotrexate (MTX)-Nitrogen mustard (HN_2) in Lymphomas. The folic acid antagonists had a limited but definite antitumor activity in the lymphomas. They were comparable in effectiveness to nitrogen mustard. Again a dose-response relationship with the antifolics was found, with the response rate increasing with dose. This indicates the lack of tumor specificity of these agents and points to their general antimetabolite action on all cells.

Comparative Studies Still in Progress:

1) Cytoxan (CY)-Uracil mustard (U.M.)-Nitrogen mustard (HN_2) study.

2) Urethane-Multiple Myeloma

3) 5-Fluorouracil (5FU), 5-Fluoro-2'deoxy-uridine (5FUDR), Methotrexate (MTX) study.

4) Parenteral Dichlormethotrexate (DCM)-Methotrexate (MTX) in Lymphomas.

5) Neuroblastoma-Chlorambucil study.

1) Cy-U.M.-HN_2: These drugs are being compared in various tumors including carcinomas of the breast, bronchus, ovary and colon and in the sarcomas and lymphomas. Preliminary results show equivalent antitumor effects with Cy and HN_2, U.M. being less effective. The gastrointestinal side effects of Cy and HN_2 are about the same and Cy has the added disadvantage of frequently causing temporary alopecia.

2) Urethane-Multiple Myeloma: Urethane is being compared to

optimal medical care (placebo). Objective tumor response has been very limited and several patients have had hepatic damage apparently induced by the drug.

3) 5FU-5FUDR-MTX: This study is primarily in patients with carcinoma of the large bowel and breast. Some antitumor effects have been seen but a high fatality rate due to the drug has lead to protocol dose modification very early in the study. The marked side effects of these agents even in "good risk" patients has raised the question whether they are generally suitable therapeutic agents despite claims to the contrary by several other workers. The study will have to be carried further however.

4) Parenteral DCM-MTX: This study is in patients with Hodgkin's disease, lymphosarcoma and reticulum cell sarcoma. It has just been started.

5) Neuroblastoma-Chlorambucil. This is the first cooperative study in childhood tumor therapy in which our hospital has taken part. Only a few cases have been entered but considerable experience has been obtained in protocol design and the use of Chlorambucil in children.

Project Title: B. Initial Therapeutic and Pharmacological Studies of Chemotherapeutic Agents in Patients with Solid Tumors.

Objectives: 1) To obtain information on the optimum route and mode of administration, toxicity and spectrum of antitumor activity of new chemotherapeutic agents in cancer patients. 2) To explore new methods for the determination of growth rate of tumors in man. 3) To determine the mode of action of various agents. 4) Trial of new techniques for assessing the status of organ function.

Methods: In the initial toxicity and antitumor trials of an agent the basic procedures are that according to a written protocol serial observations of patient reaction, organ function and tumor growth are made as various dose levels and routes of administration are explored. When the primary limiting toxicity, optimal route and maximum tolerated dose has been determined, the drug is then given to patients with a broad spectrum of tumor types while observations are made for antitumor activity. Recent additions to this phase of chemotherapy trials have been the random assignment of cases to various dose schedules and the use of sequential analysis to determine the number of cases of any one tumor type to be studied.

Serum enzyme levels are determined as parameters of tumor growth using the usual biochemical methods.

The mode of action of various drugs are studied by assaying enzyme inhibition by the drug.

The status of patients before and after therapy is being studied by organ system functional tests including the use of bacterial endotoxin to produce a controlled stress of the bone marrow and thus assess its reserve.

Major Findings:

Studies completed - 1) Parenteral Dichlormethotrexate (DCM) and Methotrexate (MTX). Maximal tolerated doses and the predominant type of side effects (hemopoietic, gastrointestinal) were established for these agents when they were given intramuscularly. 2) Lactic dehydrogenase (LDH) and Serum Oxalacetic Transaminase (SGOT) in various cancer patients during therapy. These serum enzyme levels were followed during therapy. A statistically significant correlation between changes in enzyme level and corresponding change in tumor growth rate was found for LDH. A much less close correlation was found for SGOT. The LDH may show a significant lowering as tumor regression occurs even when the starting level was within normal limits.

Studies in progress:

1) The cite of action of Azauridine is being studied in leukocytes from patients with leukemia under treatment with Azauridine. The inhibition of orotidylic decarboxylase has been demonstrated in these leukocytes. Azauridine has also induced short term decreases in the white blood count in patients with chronic myelogenous leukemia.

2) Endotoxin Bone Marrow Reserve Test: Bacterial endotoxin administered in minute doses causes a transient leukocytosis in patients with normal bone marrow function. This rise in white blood count is much less when the bone marrow has been damaged. There seems to be some correlation between the bone marrow function as indicated by the degree of leukocytosis after endotoxin administration and the patient's ability to tolerate a chemotherapeutic agent which causes hemopoietic depression.

3) Toxicity studies with Vincaleukoblastine, Epoxypiperazine and Alanine Mustard. These studies are still in progress and observations are not completed.

Significance to Cancer Research: A necessary preliminary to a clinical trial of a promising agent is a determination of its route of administration, primary side effects and probable optimum dose range.

Indirect methods of following tumor growth rates would facilitate the trial of new agents in patients in whom the neoplasms are not directly measurable; this is the case particularly for many tumors arising from the gastrointestinal tract.

As with the comparative clinical trials continuing efforts are being made to improve our methods of conducting these preliminary studies.

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Individual Project Report
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Publication:

1. Frei III, E. and Brindley, C.O. Chemotherapy of Childhood Tumors. Medical Annals of the District of Columbia. 29:257, 1960.

2. Shaw, R., Moore, E., Mueller, D., Frei III, E. and Watkin, D. Effect of Actinomycin D on childhood Neoplasms. AMA Journal of Dis. of Children 99:628, 1960.

3. Shaw, R.K., Shulman, R.N., Davidson, J.D., Rall, D.P. and Frei III, E. Studies of the Experimental Antitumor agent 4-aminopyrazalopyrimidine. Cancer 13:482, 1960.

Serial No.NCI 5-737 (c)
1. General Medicine Branch
2. Chemotherapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.:

Project Title: Infection, Immunity and Genetic Studies in Malignant Neoplastic Disease

Principal Investigators: Emil Frei III, Paul Carbone and Jacqueline Whang

Other Investigators: R. Mechanic, John P. Utz and John Fahey

Cooperating Units:
1. Laboratory of Clinical Investigation, NIAID
2. Clinical Pathology
3. Metabolism Service, NCI
4. Laboratory of Pathological Anatomy
5. Lewisburg Federal Prison

Man Years (Calendar Year 1960): Patient Days (Calendar Year 1960):
Total: 4
Professional: 2
Other: 2

Project Description:

Objectives: 1. To study the effect of malignant disease on the host as regards resistance to infection.

2. To explore the effects of malignant disease on cellular and humoral factors of resistance.

3. To study chomosomal morphology and number of malignant cells with relation to clinical and laboratory variables.

Methods: Case Material - All patients on the Chemotherapy Service are potential candidates for these studies.

Laboratory - The pathological, serological and bacteriological, biochemical methods are standard except as specifically qualified below.

Major Findings:

1. Acute Leukemia

The results of these studies have been presented in previous Project Reports and are in references 1, 2, 3, and 4.

2. Chronic lymphatic leukemia. The results are as follows: (a) Circulating antibody production is depressed and correlates generally with the degree of hypogammaglobulinemia. (b) The frequency of infections

correlates inversely with ability to produce circulating antibodies. (c) Cellular antibody production as measured by delayed skin sensitivity is normal. (5)

Prednisone increases the frequency and particularly the severity of infections in chronic lymphocytic leukemia. Prednisone produces a brief and discordant antileukemia effect and therefore should be used only for symptomatic hemolytic anemia or when other therapeutic measures have failed (6).

3. A current and cumulative record of all infections within NCI is being maintained. Products of this effort in the past have been reports on pseudomonas and clostridial infections. Analyses of our monilial and cryptococcal infections have been prepared for publication. A comprehensive clinicopathologic correlative study of all of the systemic fungal infections within the NCI is underway.

4. Endotoxin studies

The biological effects of endotoxin administration are being increasingly defined tho many facets remain controversial. One of these is the evidence that the increase in circulating granulocytes following endotoxin administration could serve as a measure of bone marrow function, specifically the marrow granulocytic reserve. In addition to the theoretical implications a simple method of measuring marrow function would be of enormous practical value as most of our therapeutic approaches to cancer involve myelosuppressive agents. This project has been approved by the Clinical Research Committee of the NIH and involves the following:

(a) Study of dose-response (WBC changes and fever) of the endotoxin Pyresal in prisoner volunteers. Preliminary results indicate that dose-response correlation is excellent and that for a given dose the variation in WBC rise is small.

(b) Small doses (0.01 - 0.025 micrograms) produce adequate increase in granulocytes with little or no fever or other morbidity. Moreover tolerance does not develop at these small doses so that the test can be repeated in the same patient.

(c) The granulocyte response in patients has been more variable and would appear to correlate with marrow function as assessed by response to subsequent administration of myelosuppressive agents. These latter studies are being extended. Response to myelosuppressive agents is probably the best measure of marrow function currently available and the degree to which it correlates response to endotoxin stimulation will be critically evaluated.

5. Cytogenetic Studies. Cytogenetic studies of clinical material will almost certainly expand enormously at NCI. The method for study of

chromosomal morphology and enumeration has been refined and simplified by Whang and Tjio and can thereby be applied on a fairly large scale to clinical material. Emphasis will initially be placed on patients with acute leukemia. In the small number of patients examined to date abnormalities in chromosomal number have been found. The correlation of these findings with clinical manifestations, disease duration, drug resistance, etc. will be of great interest.

Proposed Course of Project

1. The clinical and pathologic scrutiny of infections in cancer and leukemia patients will continue. Major emphasis will be given to fungus infections in the next year.

2. Continued evaluation of the endotoxin stimulation text as a measure of marrow function will be made. The use of this approach to increase the yield of granulocytes and platelets for transfusion studies is underway.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Boggs, D. R. The Cellular Composition of Inflammatory Exudates in Human Leukemias. Blood 15:466, 1960

2. Boggs, D. R. and Frei III, E. Clinical studies of Fever and Infection in Malignant Neoplastic Disease. Cancer In Press

3. Boggs, D. R., Frei III, E., and Ziert, J. A Controlled Study of Tetracycline in Fever of Malignant Neoplastic Disease. Annals of Internal Medicine 53:754, 1960

4. Silver, R. T., Utz, J. P., Fahey, J. L. and Frei III, E. Antibody Response in Patients with Acute Leukemia. J. Lab. & Clin. Med. 56:332, 1960

5. Shaw, R, Szwed, Clarence, Boggs, Dane R., Fahey, John L., Frei III, Emil, Morrison, Eleanor and Utz, John P. Infection and Immunity in Chronic Lymphatic Leukemia. Archives of Int. Med. 106:467, 1960

6. Shaw, R., Boggs, D. R., Silberman, H. and Frei III, E. A controlled study of Prednisone Therapy of Chronic Lymphatic Leukemia. Blood In Press.

Serial No. NCI 5 739 C
1. General Medicine Branch
2. Chemotherapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Endotoxin Stimulation Test as a Measure of Bone Marrow Function

Principal Investigators: Emil Frei III

Other Investigators: M. Landy and R. Mechanic

Cooperating Units: Lewisburg Federal Prison

Project Description:

This project is included with NCI 5 737 (c). Thus Project #NCI 5-739 C is terminated.

Serial No. NCI-1-223(c)
1. GMB
2. CP & ET
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Penetration of Antitumor Drugs into the Central Nervous System

Principal Investigators: David P. Rall, Charles G. Zubrod

Other Investigators: None

Cooperating Units: National Institute of Mental Health, M. PA 20 (Dr. Streicher).

Man Years (calendar year 1960): Patient Days (calendar year 1960):
Total: 2 1/2 0
Professional: 1 1/2
Other: 1

Project Description:

Objectives: The objectives of this project are to quantify the penetration of certain antitumor drugs into the central nervous system (CNS) and to attempt to elucidate some of the factors influencing the relationship between dose of drug, plasma level, and concentration of drug in the CNS.

Methods: Standard pharmacologic technics were used in these studies.

Major Findings: The studies of this year have been of an exploratory nature focused on the basic problem of why certain compounds do not enter the cerebrospinal fluid from blood. The observations reported last year that sulfanilic acid is relatively excluded from the spinal fluid of the dog fish for 100 hours, but that paraminohippurate does enter the spinal fluid, with a half time of 50 hours form the basis for many of this years experiments. The exit rates of both of these compounds have been determined following their intraventricular administration into the dogfish. Although the half time for entry of these compounds is in the order of fifty hours, the exit rate of these

Part B included Yes

compounds is approximately 1 hour, with sulfinilic acid leaving more rapidly than paraminohippuric. Some evidence has been obtained that penicillin, a strong acid, can slow down the exit rate of both these two compounds in the dogfish. The observation that sulfanilic acid is excluded from the cerebrospinal fluid even after long periods of time is evidence that there is some active process which either prevents the entry or speeds the exit of this compound. Two possibilities are either a stereo-specific active transport mechanism from cerebrospinal fluid to plasma or bulk flow of the cerebrospinal fluid removing drug and fluid at such a rate that the inward diffusion is inadequate to maintain a ratio approaching unity. In preliminary experiments in dogs, if the rate of flow of cerebrospinal fluid is diminished by the administration of ascedizolamide, a carbonic an hydrase inhibitor, certain compounds such as inulin which enter CSF to a limited extent have been shown to enter CSF more readily than normal. This provides evidence in favor of the bulk flow hypothesis.

In a collaborative study with Dr. Streicher, it has been shown that the CSF/Pl ratio for thiocyanate is directly related to the plasma thiocyanate concentration. At plasma thiocyanate concentrations of 8-10 mM/L the ratio approaches unity, while at plasma concentrations of 1-2 mM/L the ratio is about 0.1. This again indicates that some active process must be involved.

Studies with glucose in the dog in terms of the CSF: plasma ratio of glucose in the normal, hypo- and hyperglycemic animal have indicated that the CSF plasma ratio remains reasonably constant at about 80 per cent. Glucose was measured by the specific glucose oxidase method. Insulin infusion or glucose infusion were used to alter the plasma glucose level. The implication of the finding that the ratio remained less than unity in spite of such manupulation is that some sort of active process is involved in glucose exchange.

Techniques have been worked out to allow repeated sampling of spinal fluid plasma in dogs maintained under anesthesia for periods as long as 48 hours. This has been done studying tetracycline and dimethylchlortetracycline. For both of these compounds entry in spinal fluid after the first 12 hours is only 10 per cent. The CSF concentrations after 36 hours are only about 30 per cent of the plasma value. Similar studies in nephrectomized dogs are being performed with C^{14}-labeled dextran of varying but known and homogeneous molecular size. These compounds, with molecular weights of 12 thousand and of 77 thousand, equilibrate after approximately 24 to 36 hours with a plasma CSF ratio of 100 to 200 to 1. Analysis of this data should indicate the magnitude of the pores which exist between plasma and cerebrospinal fluid.

Studies on protein binding of drugs as this affects entry into cerebrospinal fluid as well as other tissue and fluid compartments led to the observation that a new plasma protein component appears with metamorphosis in the sea lamprey (Petromizon Marinus Dosatus). On paper strip electro-

phoresis this component migrates at a faster rate then any of the components present in the larval form. Its rate is still slower than that of human albumin. Along the same lines we have studied dogfish plasma protein and obtained evidence that there is no albumin in the plasma of this species.

In a brief exploratory study a method was devised for estimating triethanolamine, a weak base with a pK of approximately 7.5, which would be extremely useful in determining the intracellular pH of various normal and neoplastic cells. The technique which was devised was adequate to demonstrate that this is an excellent compound for this purpose, but is to cumbersome for routine use. The availability of isotopically labeled triethylolamine is being investigated.

Significance to Cancer Research: Tumors, both primary and metastatic, frequently develop within the central nervous system. Little information is available in the published literature concerning the penetration of antitumor drugs into the CNS. Basic research on this aspect of the pharmacology of antitumor drugs is necessary for the rational use of these agents in the treatment of patients with tumors located in and around the brain.

Proposed Course: The following lines of investigation are planned:

1. Further attempts to elucidate some of the basic pharmacologic factors which determine both how much, and how fast drugs get into the CNS and CSF from plasma. These include cerebral blood flow, state of thyroid function, etc.

2. Continued investigation of the comparative pharmacology of CNS and CSF. The technical ease with which the ventricular fluid and brain are obtained in dogfish make this species particularly valuable.

3. Studies in patients with suitable neoplasms will be continued, and extended to include the use of antitumor drugs.

4. Studies with compounds which may enter or leave the brain and CSF by stereo-specific transport mechanisms.

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Part B

Rall, D. P., Schwab, P. and Zubrod, C. G. Alteration of Plasma Protein Components During Metamorphosis of the Sea Lamprey (Pteromyzon Marinus Dosatus). Science, in press.

Rall, D. P., Moore, E., Taylor, N. and Zubrod, C. G. A Study of the Blood-Cerebrospinal Fluid Barrier in Man. Arch. Neurol., in press.

Serial No. NCI-1-225(c)
1. GMB
2. CP & ET
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Pharmacology of Antitumor Agents Related to Purine

Principal Investigator: Ti Li Loo

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1/2
Professional: 0
Other: 1/2

Project Description:

Objectives: To study the fate and metabolism of certain antitumor agents of the purine group currently in use.

Methods: Standard chemical and biochemical methods were employed.

Major Findings: Owing to more pressing problems connected with other investigations, this project has been dormant most of the time. However, the intrathecal administration of 6-mercaptopurine riboside was studied. Toxic but nonlethal levels were determined. The CSF concentration of the drug depended on the dose; they usually reach a low level after 4 hours and the rate of decrease followed first order diffusion kinetics.

Significance to Cancer Research: The studies of the metabolic fate of antitumor agents are important because of the light such studies could throw upon the mode of action of these agents. Further, the mechanism of the development of drug resistance could also be partly elucidated through the metabolic studies.

Proposed Course: Continue the isolation and identification of all metabolites of 6MP riboside in both responsive and resistant patients.

Part B included No

Serial No. NCI-1-226(c)
1. GMB
2. CP & ET
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Metabolism of Folic Acid Antagonists

Principal Investigators: Vincent T. Oliverio, Ti Li Loo

Other Investigators: J. D. Davidson, R. Dion, D. Rall

Cooperating Units: Chemotherapy Service, GMB, NCI

Man Years (calendar year 1960):
Total: 6
Professional: 3
Other: 3

Project Description:

Objectives: To study the total pharmacology of certain folic acid antagonists with the view to elucidating their mode of action.

Methods: Analysis of patient urine, as well as urine, blood, bile, cerebrospinal fluid and feces of animals following treatment with methotrexate (MTX) or dichloromethotrexate (DCM). Assays involving standard microbiological techniques, an improved fluorometric procedure, and isolation of the drugs and their metabolites by newly developed ion-exchange column techniques. Identification and confirmation of compounds by UV spectral analysis, paper chromatography, high voltage electrophoresis, comparative column chromatography and comparison with synthesized authentic compounds. Radioactive assays have been done with liquid scintillation counting and autoradiography.

Major Findings: The study of the physiological disposition of several folic acid antagonists of current interest in cancer chemotherapy necessitated the development of generally applicable methods for the quantitative separation and analysis of mixtures of closely related folic acid analogs. This has been accomplished by the use of

Part B included Yes

linear gradient elution with pH 8 phosphate buffer from DEAE ion-exchange columns. More than fifteen closely related folic acid analogs, were resolved quantitatively in both micro and preparative scales from various admixtures.

Application of this method to the recovery of methotrexate (MTX) and dichloromethotrexate (DCM) from patients urine revealed that only 40-50 per cent of a parenterally administered dose is excreted and this occurs within 24 hours. The urine of DCM-treated patients contains a metabolite, which constitutes one fifth of the drug administered. By contrast, the urinary recoveries of either MTX or DCM in patients even after a large oral dose were extremely low.

DCM labeled with radioactive chlorine was synthesized. This is the first radioactive antifolic to be available in adequate amount and purity for pharmacological studies. Quite unexpectedly, this compound proved to be extensively excreted in the bile in rats, mice and dogs. It concentrates rapidly in the liver and persists there for more than a week after a single dose. The DCM metabolite occurred in human, rat and mouse urine and in the bile of rats but not of dogs. In mice, 90 per cent of orally administered DCM was found in the feces.

On chemical and spectrophotometric grounds, it was predicted that the DCM metabolite was a 4,7-dihydroxy derivative. The identity of the metabolite has been confirmed by chemical synthesis and comparison of physical properties.

The study of the metabolism of DCM was extended to include biological halogenation and dehalogenation in view of the possibility of *in vivo* conversion of DCM to MTX. Enzymatic halogenation by a chloroperoxidase system isolated from mycelia of *Caldariomyces fumago* was consequently investigated. It was shown that the chlorination and also bromination of MTX could be brought about by this interesting enzyme system.

By the published technique of the fluorometric assay, quantitative recoveries of DCM from plasma have thus far been erratic. A new method of protein precipitation using a mixture of dimethylformamide and chloroform has been developed. This is then converted to a biphasic system with additional chloroform, and the aqueous phase, is treated in the usual manner. Plasma recoveries with this technic are essentially 100 per cent.

Plasma protein studies indicated that DCM, at therapeutic levels, was 80-85 per cent bound, both in dogs and humans. Subsequent stidues with purified bovine and human serum albumin gave the same degree of binding. The number of binding sites appears to be approximately 10, presumably amino groups, and the association constant for the DCM-albumin complex approximately 1.0×10^3.

The plasma half time for DCM in man after intravenous injection was about 3 hours, somewhat longer than for MTX. DCM was distributed in 20-30 per cent of body weight. Very low plasma concentrations of DCM were observed after oral drug administration.

Studies on the histology of the small intestine of man after large doses of folic acid antagonists are in a preliminary stage, but indicate that mitotic activity was arrested within 6 hours, and resumes some 66 hours later.

Significance to Cancer Research: The studies of the metabolic fate of antitumor agents are important because of the light such studies could throw upon the mode of action of these agents. Further, the results of such studies may serve as an intelligent guide to the design and synthesis of potential antitumor agents in the future.

Proposed Course:

1. Continue pharmacological studies on DCM in animals and extend to patients using both labeled and unlabeled drugs.

2. Prepare compounds related to DCM metabolite for screening and pharmacological studies.

3. Prepare C^{14}-methotrexate for tracer studies in animals and man.

4. Improve present techniques for study of DCM and MTX pharmacology.

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Part B

Oliverio, V. T. Chromatographic Separation and Purification of Folic Acid Analogs. Anal. Chem., in press.

Dion, R. and Loo, T. L. The Oxidation of Certain Folic Acid Antagonists. J. Org. Chem., in press.

Serial No. NCI-1-227
1. GMB
2. CP & ET
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Mechanism of Antitumor Action of Purine Analogs

Principal Investigator: Jack D. Davidson

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1 1/2
Professional: 1
Other: 1/2

Project Description:

Objectives: The objective of this project is to determine as specifically as possible which biochemical reactions are inhibited by certain purine analogs in cancer cells that are sensitive to these analogs. A corollary objective is to elucidate the mechanisms responsible for inherent and acquired resistance to these agents.

Methods: Radioactive purines, purine precursors, and purine analogs were used with both intact cells and isolated enzyme systems to study the effects of the analogs on purine metabolism and to investigate the metabolism of the analogs. New techniques include improved methods for isolating leukocytes from human blood, improved high voltage paper electrophoresis for the isolation of purine moieties from small samples of cells, and a precise method of liquid scintillation counting of samples on electrophoresis papers.

Major Findings: Previous work on the mechanisms of action and resistance of 6-mercaptopurine in mouse leukemia was published. To determine whether these findings apply to human leukemia, new techniques were developed to assay inosinic pyrophosphorylase in human leukocytes. Eighteen patients with leukemia have been studied. All showed some capacity to convert hypoxanthine-C^{14} and 6-mercaptopurine-S^{35}

Part B included Yes

to nucleotides. Only four apparently 6-mercaptopurine-resistant patients have been studied and two of these had inosinic pyrophorylase levels in the "normal" range, suggesting that their resistance was not due to the deficiency of this enzyme which characterizes resistant bacteria and mouse leukemia.

In previous work on mouse leukemia, there were findings that suggested a competition between 6-mercaptopurine and hypoxanthine for inosinic pyrophorylase. This has now been confirmed by studies with the isolated enzyme.

Significance to Cancer Research: Since purine analogs include some of the most effective antitumor agents thus far discovered, an understanding of the mechanisms of their action and of resistance thereto should contribute to the rational development of more effective antitumor chemotherapy.

Proposed Course:

1. Continuation of studies of inosinic pyrophosphorylase in human leukemia with emphasis upon assay of the enzyme levels in the same patient at times when his leukemia is sensitive to 6-mercaptopurine and after resistance has developed.

2. Studies on other possible mechanisms for resistance in human leukemia, such as permeability to drug, catabolic destruction of drug, and altered enzymes.

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Individual Project Report
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Part B

Davidson, J. D. Studies on the Mechanism of Action of 6-Mercaptopurine in Sensitive and Resistant L1210 Leukemia *in vitro*. Cancer Res., 20: 225-232, 1960.

Shaw, R. K., Shulman, R. N., Davidson, J. D., Rall, D. P. and Frei, III, E. Studies with the Experimental Antitumor Agent 4-Aminopyrazolo(3,4-d)-pyrimidine. Cancer, 13: 482-489, 1960.

Davidson, J. D. Some Recent Developments in Liquid Scintillation Counting of Biochemical Samples. In press. Monograph on Proceedings of the University of New Mexico Conference on Organic Scintillation Detectors, August 15-17, 1960, Albuquerque, New Mexico.

Serial No. NCI-1-229(c)
1. GMB
2. CP & ET
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Pharmacology of Riboflavin Analogue

Principal Investigator: Montague Lane

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1.0
Professional: 0.5
Other: 0.5

Project Description:

Objectives: Clinical. To induce tumor regression and/or clinical riboflavin deficiency in patients with neoplastic diseases through the use of riboflavin analogues.

Methods: Spectrophotometry, photofluorometry, chromatography, bio-assay and isotope (Fe^{59}) techniques were used.

Major Findings: Clinical. Riboflavin deficiency has been induced with the riboflavin antagonist, galactoflavin, and a semi-synthetic diet complete in nutrients but lacking riboflavin, in a total of four patients with different malignant diseases. Signs of deficiency have included seborrheic dermatitis of face, nose, abdomen, scrotum and legs, glossitis, angular stomatitis, and cheilosis.

In addition, each patient developed reticulocytopenia and anemia, which appeared to be normochromic and normocytic. Upon addition of riboflavin to the regimen, a reticulocytosis promptly occurred. A role can now be ascribed to riboflavin in erythropoiesis in man.

Studies of urine riboflavin showed that a marked increase in riboflavin excretion, above dietary intake, accompanied the administration of gal-

Part B included Yes

actoflavin. This demonstrates that galactoflavin is a riboflavin antagonist in man and displaces riboflavin from various sites in the body.

Significance to Cancer Research: Riboflavin analogues have been shown to inhibit rat tumor growth. The development of a regimen which will result in clinical riboflavin deficiency may permit the evaluation of the effects of this deficiency on the growth of tumors in man. Combination chemotherapy studies may also be feasible. In addition, a system is provided for the further elucidation of the physiological role of riboflavin in man.

Proposed Course: This investigator has left the National Institutes of Health; therefore, this project is being closed out.

Part B

Lane, M. The Effectiveness of Cyclophosphamide (Cytoxan) Against Well Established Transplanted Rodent Tumors. Cancer Res. 20: 1269-1273, 1960.

Lane, M. and Kelly M. G. The Antitumor Activity of 5-Bis-(2'-chloroethyl)-Aminouracil (Uracil Mustard). Cancer Res. 20: 511-517, 1960.

Serial No. NCI-1-231(c)
1. GMB
2. CP & ET
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on Tetracyclines

Principal Investigator: Kurt W. Kohn

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):
Total: 2
Professional: 1
Other: 1

Project Description:

Objectives:

1. To develop a method for the determination of tetracyclines in tissues, which would be independent of the problems inherant in bioassays.

2. To determine the volumes of distribution, plasma protein binding, and extent of entry into spinal fluid, red blood cells, and skeletal muscle of tetracycline and demethylchlortetracycline in anesthetized dogs.

3. To study the binding of tetracyclines to small molecules, nucleic acids, and protein.

Methods: The methods employed were solvent extraction using complex systems, equilibrium dialysis, fluorescent and spectrophotometric measurements and the usual pharmacologic technics.

Major Findings: The extraction-fluorescence method for tetracycline and chlortetracycline, as developed last year was compared with the standard microbiological assay method. The agreement was very good; the chemical method seemed to be more precise.

Part B included Yes

The findings of last year were confirmed and extended. Tetracycline and dimethylchlortetracycline were found to be concentrated in muscle 1.5-4 times over plasma. Other tissues; thyroid, lung, etc. seemed to be approximately in diffusion equilibrium with plasma as far as both drugs are concerned.

Equilibrium dialysis experiments showed that tetracycline did bind to deoxynucleic acid of salmon sperm.

Significance to Cancer Research: Tetracyclines exhibit the ability to concentrate selectively in certain tissues, including some tumor cells. This binding may be a tool for improving our knowledge of the composition or structure of tumor cells under various conditions.

An understanding of the factors which govern the cellular distribution of these drugs may be of general value in chemotherapeutic problems. The tetracyclines are also of interest because of their antibiotic properties. Elucidation of the mechanism in this instance of selective cellular toxicity would be of vital interest.

The present studies on the complexing properties of tetracyclines are preliminaries aimed at these goals.

Proposed Course: The principal investigator has left for a period of out of service training. This project is, therefore, being closed out.

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Individual Project Report
Calendar Year 1960

Part B

Kohn, K. W. Determination of Tetracyclines by Extraction of Fluorescent Complexes. Application to Biological Materials. Anal. Chem., in press.

Serial No. NCI-1-232
1. GMB
2. CP & ET
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Pharmacology of Drugs Influencing the Response of Radiomimetic Drugs and Radiation

Principal Investigator: Margaret G. Kelly

Other Investigators: R. W. O'Gara and T. L. Loo

Cooperating Units: Laboratory of Pathology and Radiation Branch

Man Years (calendar year 1960):
Total: 1/2
Professional: 1/4
Other: 1/4

Project Description:

Objectives:

1. To study the mechanism of protective effect of AET on toxicity of nitrogen mustard in normal and tumor bearing mice and rats.

2. To determine localization of C-14-labeled AET in tissues and cells by application of autoradiogram techniques.

3. To evaluate protective effect of other classes of compounds (in addition to mercaptoalkylguanidines) against radiomimetic drugs and radiation, and to explore combinations of protective agents.

Methods: Usual pharmacologic, hematologic, chemical, biologic, histopathologic, and radioactive tracer techniques.

Major Findings:

1. Microautoradiograms have been made with C-14-labeled AET, and the cellular localization of AET has been studied. Apparently, the label is distributed fairly uniformly throughout the cyto-

Part B included Yes

plasm, and there is no evidence of intranuclear incorporation. As reported last year, AET concentration (as measured by C-14) is low in tumor implants and in metastatic foci from primary implants.

2. Studies on toxicity of compounds which generate free radicals have been continued, particularly with di-t-butylperoxide and t-butylperbenzoate. The toxicity of these 2 compounds has been determined in mice and rats. One of the problems with these compounds is that they are insoluble in aqueous solution and are not miscible with water. However, they can be given as an emulsion (not very stable) in methocel, or in olive oil, with which they are miscible. Intraperitoneal administration of either di-t-butylperoxide or t-butylperbenzoate 30 minutes before intraperitoneal administration of HN2, or before whole body radiation decreases the lethality. The optimum protective effect has not been determined as yet, but the data suggest about a 50 per cent increase in LD_{50}. Neither compound is particularly effective by oral or subcutaneous route.

   Pretreatment with di-t-bytyperoxide or t-butylperbenzoate does not appear to block the therapeutic effect (increase in survival time) or HN2 in rats bearing subcutaneous implants of Dunning leukemia, and at some dose levels of HN2 the therapeutic effectiveness of HN2 may, perhaps, be increased.

Significance to Cancer Research: Chemical protection against systemic toxicity of alkylating agents or radiation without comparable protection of tumor against their carcinoclastic action would result in a therapeutic advantage.

Proposed Course: AET labeled with tritium will be used to get additional information on cellular localization, particularly within the nucleus.

1. To date, all our data on distribution of AET in tumor tissue has been obtained with transplanted tumors. This work is being extended to include the distribution of C-14-labeled AET in induced tumors. It is possible that the failure of AET to localize in tumor implants may only reflect failure of the compound to reach the tumors because of the relatively new vascular supply to the implant and may not represent the distribution of AET in tumors arising in situ.

   As mentioned last year, a comparative study of the urinary excretion products of C-14-labeled AET in dogs and mice was planned in collaboration with Drs. Claudia Prichett and P. K. Smith, George Washington University Medical School. They have

furnished us with the labeled AET used in the past, and have studied the urinary metabolites in man. It is hoped that this study will be made in the near future as it may help explain why AET is so much more toxic for man, and dog than for mice, and why it gives man and dog no protection from either HN2 or radiation.

2. To continue studies on the protective effect against radiation and alkylating agents of free radical generating compounds in normal and tumor bearing mice and rats, and to determine the toxicity and protective effect of these materials in dogs.

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Individual Project Report
Calendar Year 1960

Part B

Kelly, M. G., Rall, D. P., Trivers, G. E., O'Gara, R. W., and Zubrod, C. G. Actions of S,2-Aminoethylisothiuronium Br. HBr (AET). Toxicity and Protective Effect Against Nitrogen Mustard Toxicity. J. Pharm. and Exper. Therap. 129: 218-230, 1960.

Serial No. NCI-1-233
1. GMB
2. CP & ET
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Chemical Carcinogenesis

Principal Investigator: Margaret G. Kelly and Roger W. O'Gara

Other Investigators: None

Cooperating Units: Laboratory of Pathology, National Cancer Institute

Man Years (calendar year 1960):
Total: 2.5
Professional: 1.5
Other: 1

Project Description:

Objectives: To determine the effect of chemical carcinogens in newborn animals.

Methods: Usual biologic and histopathologic techniques.

Major Findings:

1. Studies covered in last year's report have been completed and may be summarized as follows: Multiple tumors of the lung, subcutaneous fibrosarcomas, leukemia and hepatomas have been induced within 8 to 32 weeks following a single, subcutaneous injection of 0.06 mg dibenzanthracene or 0.1 mg 3-methylcholanthrene into newborn mice (non-inbred albino, C3H/P, C57 BL/-6JN). The doses employed were well tolerated. Direct comparison showed that newborn mice were more susceptible than older sucklings or adult mice to induction of pulmonary tumors. Newborn mice of the albino strain were the most susceptible, and newborn C57BL mice were the least susceptible to lung tumor induction. In albino mice treated during the first 18 hours a after birth, essentially 100 per cent of the animals developed pulmonary tumors within 16 to 24 weeks after subcutaneous administration of either 0.02 mg of dibenz(a,h)anthracene or

Part B included Yes

0.011 mg 3-methylcholanthrene, and 38 per cent of the mice developed pulmonary tumors after 0.0022 mg dibenz(a,h)anthracene. Repeated experiments have given consistent data on latent period, incidence of induced pulmonary tumors, and mean number of tumor nodules per animal. The later measurement appears to be a more sensitive index of response and/or carcinogenic potency than the incidence of mice with pulmonary tumors or the latent period. The pulmonary tumors arose from alveolar cells. Histopathologic study of these neoplasms suggested that the carcinogenic process in the lung was sustained, and that new tumors were forming as late as 24 weeks after injection of carcinogen. Subcutaneous fibrosarcomas were induced at the site of injection by both carcinogens. Study of early subcutaneous tumors demonstrated that many of these tumors arose from the perimysium of subcutaneous muscle, although they grew as spindle cells fibrosarcomas. A greater variety of tumors were induced with 3-methylcholanthrene than with dibenz(a,h)anthracene. Mice given 3-methylcholanthrene as newborns developed leukemia (albino, C57BL strains), multiple hepatomas (C3H males and females) and multiple adenomas of the sebaceous glands (albino and C3H mice). Leukemia usually occurred in 9 to 16 weeks after injection, and was rapidly fatal. The hepatomas and sebaceous gland adenomas were more benign, and were found at autopsy in mice killed at the end of the observation period (24-32 weeks after administration of carcinogen).

2. In collaboration with Mr. Mantel (Biometrics Branch) a study has been initiated (now in its 21st. week) to determine the lowest dose of dibenz(a,h)anthracene and 3-methylcholanthrene that will induce tumors in mice given a single, subcutaneous injection of carcinogen as newborns. The observation period for this study will be 48 weeks. Eight logarithmically-spaced dose levels of each drug are being tested and there are 35-60 mice per dose level. At the present time, fibrosarcomas have been obtained at 0.0002 mg DBA and 0.0004 mg MC, and lung tumors with 0.0067 mg DBA and 0.0037 mg MC. One animal developed leukemia 17 weeks after 0.0067 mg dibenz(a,h)anthracene.

3. Some studies have been made with higher dose levels of methylcholanthrene in an attempt to induce a higher incidence of leukemia and hepatoma. About 30 per cent incidence of leukemia is obtained with 0.3 mg of 3-methylcholanthrene s.c. to newborn albino mice. The peak incidence occurs in 9-14 weeks, and few animals develop this neoplasm after 16 weeks. In C3H mice given this dose, about 75 per cent of the males develop hepatomas within 32 weeks. Hepatomas were induced also in the females of this strain, but the incidence and number of foci per liver were less than in the males.

4. For results in newborn hamsters given a single, subcutaneous injection of either 0.1 mg or 0.45 mg 3-metholanthrene in 0.05 ml olive oil, see O'Gara, Laboratory of Pathology.

5. For results in albino mice given 2-fluorenylacetamide by subcutaneous, intraperitoneal, and oral routes, respectively, on the day of birth, and at 3 and 6 days after birth, see report of O'Gara, Laboratory of Pathology.

6. For results in mice given 3-amino-1,2,4-triazole continuously for 24 weeks, beginning at birth, see report of O'Gara, Laboratory of Pathology.

Significance to Cancer Research:

1. Recognition of age differences in susceptibility to chemical carcinogens may be of importance in selecting "safe limits" for exposure of human population to environmental carcinogens.

2. The greater susceptibility of newborn mice may afford a more sensitive and efficient assay system for carcinogens of low potency.

3. Recent viral studies in mice indicate that most, if not all, inbred and random bred stock mice carry viral agents. It is important, therefore, to determine whether or not this "viral background" influences tumor induction by chemical carcinogens.

4. Understanding of factors (local and systemic) responsible for increased susceptibility of newborn mice to chemical carcinogens may help elucidate the mechanism of tumor induction.

Proposed Course: A collaborative study has been initiated with Dr. Walter Newton, National Institute of Allergy & Infectious Diseases (Lab. Germ-free Animal Res.) to measure the carcinogenic effect of a single, subcutaneous injection of 3-methylcholanthrene in newborn, germ-free Swiss mice. So far as is known, these mice are also free of virus. Two dose levels have been used: 0.1 mg and 0.3 mg. It is hoped that the higher dose will furnish information on incidence of leukemia and subcutaneous fibrosarcomas as well as on pulmonary tumors. Our data on mice obtained from the N.I.H. breeding unit (random-bred albino) should furnish a good basis of comparison, and in addition other types of controls have been set up.

Effect of age on susceptibility of mice to radiation-induced leukemia is being investigated. C57BL mice have been given a single whole-body dose of 400 r (200 kv machine) either as newborns, or at 2, 4, or 8 weeks of age. In adult mice, this dose should produce about 30-40 per cent incidence of leukemia by 48 weeks after irradiation. The mortality has

been less than 10 per cent in all age groups except in the animals radiated at 4 weeks of age, in which more than 50 per cent mortality was obtained in the first 2 weeks post-radiation. As this experiment has just been initiated, no results are available at this time.

In collaboration with Dr. Vincent Oliverio, a comparison will be made of the absorption, distribution, metabolism and protein binding of dibenz(a,h)anthracene in newborn and adult mice. Dr. Oliverio has labeled dibenz(a,h)anthracene with C^{14}.

The susceptibility of newborn animals to other chemical carcinogens of varied structure will be evaluated.

The carcinogenic response of combinations of subminimal levels of carcinogenic agents (chemical, viral, radiation) will be determined. Newborns of other species than rodents (dogs, monkeys) will be given a chemical carcinogen to determine whether the increased susceptibility observed in mice is a general biologic phenomenon.

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Individual Project Report
Calendar Year 1960

Part B

Kelly, M. G. and O'Gara, R. W. Induction of Tumors in Newborn Mice With Dibenz(a,h)anthracene and 3-methylcholanthrene. J. Nat. Cancer Inst., In Press.

Serial No. NCI-1-234(c)
1. GMB
2. CP & ET
3. Bethesda

PES-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Pharmacology of Alkylating Agents and Other Antitumor Drugs

Principal Investigator: Margaret G. Kelly

Other Investigators: Dr. Roger O'Gara

Cooperating Units: Laboratory of Pathology, National Cancer Institute

Man Years (calendar year 1960):
Total: 3.5
Professional: 1 1/4
Other: 2 1/4

Project Description:

Objectives:

1. To study the pharmacology of antitumor drugs of current interest in experimental animal or clinical cancer chemotherapy.

2. To evaluate the usefulness of new tumor systems in experimental chemotherapy.

Methods: Usual biologic and histopathologic techniques. Injection of newborn animals.

Major Findings:

1. (In collaboration with Dr. Montague Lane)---Effectiveness of uracil mustard under various treatment regimens has been determined and compared with other antitumor agents against a spectrum of tumors. The results of this study have been published.

2. Use of carcinogen-induced tumors for chemotherapy studies: We have been investigating tumor induction in mice given a chemical carcinogen as newborns, and find the newborn more susceptible than older mice. The dose response has been very

Part B included Yes

good, and it has been possible to produce pulmonary tumors, subcutaneous fibrosarcomas, leukemia, and hepatomas in good incidence. The latent period and incidence for each type of tumor has stayed within fairly narrow limits, thus making feasible chemotherapeutic trial against at least 3 types of primary tumors in the same host. In the only experiment completed to date, treatment was started when the mice were 8 weeks old, and continued for 8 weeks (16 weeks old). The animals were killed 3 days after the last treatment. With the dose of carcinogen used in this experiment, multiple tumors of the lung were induced in 70 per cent of the animals by 8 weeks, and in 100 per cent by 16 weeks. By 16 weeks, also, there was about a 15 per cent incidence of fibrosarcomas and leukemia, but the trial was designed to evaluate only the effect on the pulmonary tumor incidence and nodule count.

The effect of treatment was evaluated on the following grounds:

a. Incidence or number of lung tumor nodules per mouse as compared with 16 week old controls.

b. Change in either measurement over that obtained at 8 weeks when treatment was begun.

The drugs, dose, route, schedule, etc., were as follows:

Oxytetracycline,	1% in drinking water
6-mercaptopurine,	50 mg/kg, i.p., 2 X week
Methotrexate,	10 mg/kg, i.p., 2 X week
Nitrogen mustard (HN2),	2 mg/kg, i.p., 1 X week
Cytoxan,	100 mg/kg, i.p., 1 X week
Cortone acetate.	200 mg/kg, s.c., ca. 1 X week

After 8 weeks of treatment with oxytetracycline, 6-MP, or MTX, the incidence of mice with pulmonary tumors was the same, and the number of tumor nodules per mouse was either the same or greater than that in carcinogen-control mice killed at the same time (16 weeks). At the doses used, these 3 drugs had essentially no effect on body weight, although a rather marked fall in hematocrit was produced by 6-MP. Animals given HN2, cytoxan, or cortisone showed no increase in incidence or nodule count over the 8 week treatment period, being about the same as that of the 8 week controls. Weight loss with these 3 compounds varied between 7-20 per cent.

Very little data were available concerning the toxicity of any of these drugs on administration over an 8 week period, and treatment with cytoxan and cortisone had to be interrupted because of loss of weight, death, etc. Since then, a study of toxicity has been made in normal mice in order to furnish data on toxicity of repeated administration. Histopathologic examination of tissues has not been completed, but should furnish supplementary data on the toxicity of these drugs.

3. Studies with mast cell tumors:
The mast cell tumor is of interest in experimental chemotherapy because it has a measurable content of serotonin, histamine, and a mucopolysaccharide. Preliminary studies showed that treatment of mice bearing subcutaneous implants of the solid form of this tumor (low in serotonin) with HN2 or uracil mustard prolonged survival time. Further studies with the ascitic form of this tumor (high in serotonin) have showed that survival time can be increased 100 to 200 per cent by weekly oral administration of cytoxan (179 to 444 mg per kg). Methotrexate (25.6 to 50 mg per kg s.c.) increased survival time about 100 to 30 per cent, and radiation (whole body, 300-600 r) increased survival time about 20 per cent.

Following treatment of the ascitic form of mast cell tumor with either cytoxan, methotrexate, or radiation (initiated after the tumor is well established), the ascites disappears temporarily, and the serotonin level first rises and then falls. The histamine level, however, remains fairly constant.

During the course of some 170 transplant generations, there has been a marked reduction in serotonin content of the solid tumor and an increase in the content of serotonin in the ascitic form of the tumor. This has been associated with morphological changes, and there is evidence that the cell population, particularly of the ascitic form of the tumor, is heterogeneous in terms of serotonin production. To date, a stable subline has not been established, although sublines have been obtained in which the biochemical characteristics (high or low serotonin and/or histamine content) remained constant for 6 to 8 transplant generations.

Significance to Cancer Research:

1. Pharmacologic studies in experimental animals are essential before clinical testing, and often furnish useful information to the clinician.

2. One of the difficulties in selecting drugs for clinical trial is the failure of existing animal screens (which rely on

transplanted tumors) to predict with any reliability which drug will be more useful clinically. It is hoped that the use of induced tumors may prove more useful or at least supplement the present screening systems.

3. The mast cell tumor offers a unique opportunity to follow response to treatment by precise biochemical measurement of products of the tumor itself (i.e., serotonin, histamine, mucopolysaccharide).

4. Information concerning differences in metabolic pathways of normal and malignant cells, and alterations that may be produced in them, particularly when such changes can be correlated with the morphologic characteristics of normal and neoplastic cells, will afford a greater understanding of malignant disease.

Proposed Course:

1. Studies on pharmacology of alkylating agents and other antitumor compounds will be continued.

2. Chemotherapeutic studies with carcinogen-induced tumors will be continued. An experiment has just been initiated, using a dose level of 3-methylcholanthrene which should induce lung tumors in 100 per cent of the mice in 8 weeks, and 30 per cent leukemia and 65 per cent fibrosarcomas by 16 weeks. The same drugs mentioned in this report will be used, and dose levels will be adjusted to evaluate the effect of weight loss.

3. The pattern of chemical and morphologic changes that occur in untreated mast cell tumors and in tumors under therapy will be studied. Continued attempts will be made to obtain stable sublines with different serotonin and/or histamine content. The influence of serotonin on the therapeutic effect of radiation and alkylating drugs will be evaluated by comparing the effect of treatment in tumor sublines with low and high serotonin content.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B

Lane, M. and Kelly, M. G. The Antitumor Activity of 5-Bis-(2'-chloroethyl)Aminouracil (Uracil Mustard). Cancer Res., 20: 511-517, 1960.

Serial No. NCI-1-235
1. GMB
2. CP & ET
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Preclinical Toxicology of Drugs Potentially Useful in Cancer Chemotherapy

Principal Investigator: David P. Rall

Other Investigators: Margaret G. Kelly

Cooperating Units: CCNSC - NCI, Hazleton Laboratories, Falls Church, Va.

Man Years (calendar year 1960):
Total: 1/3
Professional: 1/3
Other: 0

Project Description:

Objectives: The objectives of this project are to study, in detail, the quantitative and qualitative aspects of the toxicity of drugs potentially useful in cancer Chemotherapy in order that new drugs may rapidly but safely be brought up to the point of clinical trial.

Methods: Standard toxicological methods are employed.

Major Findings: The studies this year have been concerned with the toxicology of the halogenated derivatives of Methotrexate, cyclophosphamide (Cytoxan), 5-flurouracil, nitrogen mustard, and thioTEPA. The original observation that the toxicity of dichlormethotrexate was considerably lower when given orally to rats and dogs reported her two years ago led to extensive laboratory and clinical studies on the absorption, distribution and efficacy of the folic acid antagonists as a function of the route of administration. In this service, in another projectreport, some of the findings which tend to explain this difference are being reported this year. In this toxicological project, it has been attempted to delineate under what conditions in rats and dogs the difference between oral and parenteral toxicity can be observed. The data can

Part B included No

be summarized as follows. The larger the actual dose of the compound the greater the difference between oral and parenteral administration. This has been studied in different dose schedules in rats and in dogs.

Another important aspect of clinical chemotherapy is the attempt to control meningeal leukemia. For this purpose 6-mercaptopurine riboside, aminopterin and Methotrexate have been tested for their intrathecal toxicity in dogs. 6-mercaptopurine riboside appears to be a more desirable compound than 6-mercaptopurine because of its solubility in the neutral cerebrospinal fluid. At doses of 7 and 14 mg/kilo given intrathecally into the cisterna magna there are no immediate or delayed central nervous system effects. However, as with 6-mercaptopurine, the systemic absorption of this compound leads to liver damage as evidenced by elevated BSP retention, by an increased alkaline phosphatase and serum transaminase.

The new halogenated Methotrexate derivitives, monobromomethotrexate and bromochloromethotrexate have been examined for their toxicity in rats and dogs. The monobromomethotrexate qualitatively and quantitatively resembles the monochloro- the bromochloro- similarly resembles the dichloromethotrexate.

Considerable effort has been expended this year in furthering the observation reported last year that a priming dose of Methotrexate results in a state of resistence in rats to a subsequent normally supralethal dose of Methotrexate. This observation itself has been confirmed in three separate experiments. This resistence occurs when a dose of Methotrexate which will kill ten per cent of the animals is used as the priming dose. It also occurs when a dose that will kill 1 per cent of the animals is used as well as a dose which is not lethal. In these three instances, a greater than a two fold increase in the LD^{50} was observed approximately 1-3 weeks after the priming dose. The clinical implications of this possibility of using interrupted therapy are obvious. Results with cyclophosphamid in this type of experiment have been variable. In one instance good resistence was obtained, in another experiment no resistence was obtained.

Of importance to the clinical chemotherapy groups were observations concerning the relative toxicity of the supplies of nitrogen mustard and thiotepa that have been used in a cooperative clinical study in the Eastern Solid Tumor Group. Some of the cooperating units kept the thioTEPA at room temperature, others at low temperature. The rat toxicity of the thioTEPA kept at room temperature was significantly lower then that of other samples, indicating deterioration and loss of potency. Procedures are now being worked out to prevent the recurrance of this.

The solvent for uracil mustard, N,N-Dimethylacetamide, was shown to have significant toxicity in dogs.

Previous reports have noted the striking sex difference in the toxicity of rats of acetoxycyclohexamide (E-73). Two very closely related compounds, cyclohexamide and 5-hydroxycyclohexamide do not show any sex difference in rats.

Significance to Cancer Research: The drugs currently useful in the treatment of human neoplasms must be given at near toxic doses to achieve therapeutic results. Therefore, even small differences in toxicity related to sex, age, route of administration and dose schedule, can be of considerable importance in clinical chemotherapy since this may lead to larger doses safely given to man. Information for preclinical toxicology is quickly made available to the Cooperative Groups of the CCNSC.

Proposed Course: It is planned that this project will be continued along the same lines as above. Drugs showing promise in quantitative animal tumor studies, or drugs obtained from CCNSC will be put into this toxicological appraisal before deciding on the dose, route and schedule of administration in man.

Serial No. NCI-1-236
1. GMB
2. CP & ET
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Effect of the Hypermetabolic State in Malignancy on Metabolism of Thyroxine and Hydrocartisone

Principal Investigator: Leonard Garren

Other Investigators: None

Cooperating Units: Jack D. Davidson, No. NCI-1-227; Mortimer Lipsett, No. NCI-2-803 cl

Man Years (calendar year 1960):
Total: 1/2
Professional: 1/2
Other: 0

Project Description:

Objectives: To determine whether the hypermetabolic state which occurs in the course of certain malignant diseases influences the metabolism of thyroxine and hydrocortisone as does the hypermetabolism due to hyperthyroidism.

Methods: I^{131}-thyroxine and C^{14}-cortisol were injected into hypermetabolic patients and the rates of disappearance of these hormones were determined. Studies were repeated following therapy of the malignancies and the return of the patients to the eumetabolic state.

Major Findings: Cortisol studies were completed on six patients with hypermetabolism associated with leukemia. These patients showed increased excretion of cortisol along with normal or diminished metabolism of the hormone. This contrasts with the diminished excretion and accelerated metabolism of cortisol which are found in patients with similar hypermetabolism due to hyperthyroidism. In five of the six patients, there was a return of cortisol metabolism and excretion toward normal when the basal metabolic rate returned to normal following treatment.

Part B included Yes

Significance to Cancer Research: The findings with respect to cortisol suggest that the hypermetabolic state in leukemia differs from that in hyperthyroidism and indicate that it may be incorrect to apply inferences from the latter condition to the situation of hypermetabolism in leukemia.

Proposed Course:

1. This project was terminated June 30, 1960 when Dr. Garren completed his appointment at the N.I.H.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B

Conney, A. H. and Garren, L. Contrasting Effects of Thyroxin on Zoxazolamine and Hexabarbital Metabolism. Biochem. Pharmacol., in press.

Serial No. NCI-1-237
1. GMB
2. CP & ET
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Biochemical and Pharmacological Studies of Human Neoplasms in Tissue Culture

Principal Investigator: William C. Mohler

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960): Patient Days (calendar year 1960):
Total: 1
Professional: 1/2
Other: 1/2

Project Description:

Objectives:

1. To employ tissue culture techniques

   a. for studying the mechanism of action of cytotoxic agents on animal cells.

   b. for the study of biochemical variation in human neoplastic cell populations.

   c. to develop a test of a given patient's tumor to selected therapeutic compounds.

Methods: Standard disperse cell culture techniques are modified as required.

Major Findings: Preliminary results have been obtained concerning the cytotoxicity of selected agents on established cell lines, Hela and V79.

Part B included No

A bias (10per cent or larger) has been discovered in parameters calculated from certain conventional techniques for sampling cell suspensions. Means of minimizing the bias are being worked out.

Significance to Cancer Research: In vitro cell culture techniques afford a means of examining 1) the variables controlling the growth of normal and neoplastic tissues and 2) the conditions providing maximal inhibition or labeling of neoplastic cells. So-called plating techniques provide a means of analyzing the kinetics of drug action on "pure" cell stains or mixtures of all types. Such studies should afford insights into the mechanism of action of drugs and may afford a basis for modification of treatment schedules in man. When combined with histochemical or nutritional techniques these methods should make possible the analysis of cellular population dynamics during the natural history of neoplasms and following their treatment.

Proposed Course:

1. Study of the action of selected drugs on some established cell strains in order to develop methods and gain a knowledge of the kinetics of drug action.

2. Grow cells from selected tissues with special attention to methods for obtaining a high recovery of viable cells from biopsy material (including blood cultures).

3. Study histochemical methods for identifying cell types in culture.

4. Attempt "clinical tests" of the sensitivity of biopsies of human neoplastic tissues to selected chemotherapeutic agents.

Serial No. C-7-708(c)
1. General Medicine Branch
2. Dermatology Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Clinical Investigations of Malignant Skin Diseases and of Benign Conditions Related Thereto.

Principal Investigator: E. J. Van Scott, M. D.

Other Investigators: Richard P. Reinertson, M. D., Arthur Z. Eisen, M. D.

Cooperating Units: Chemotherapy Service, NCI
Arthritis and Rheumatism Branch, NIAMD

Man Years (calendar year 1960): Patient Days (calendar year 1960)

Total:	2
Professional:	2
Other:	0

Project Dexcription: Continuation of C7-708(c) of 1959

Objectives: To study malignant processes in the skin and to carry out investigative therapy of cutaneous malignant conditions; to study benign hyperplasia of the epidermis in relation to malignant hyperplasia of the skin and other organs.

Methods Employed:

Mycosis fungoides: This disease is treated with chemotherapeutic drugs given systemically and with electron or X-radiation when indicated, and is further studied and treated by other means derived from observations on the immunologic behavior of the disease.

Basal cell carcinoma: Severe cases are treated with chemotherapeutic drugs given systemically, with X-rays, and with excision. The nature of the tumor is investigated in the patient by studying changes in the tumor occurring in response to locally administered agents and to alterations in the stromal environment of the tumor.

Psoriasis: Severe cases with and without arthritis, are treated experimentally with chemotherapeutic drugs either applied topically to the skin or given systemically. The nature of the disease, the cutaneous lesion and uric acid metabolism in psoriasis are studied in the patient.

Patient Material: Patients of the Dermatology Service, NCI
Patients of the National Institute of Arthritis and Metabolic Diseases.

Major Findings:

Mycosis Fungoides: Another chemotherapeutic drug, CYTOXAN, has been found to have beneficial influence on this disease. In one of five patients studied the use of the drug has been followed by complete disappearance of tumors from the skin. Continued administration of the drug has been necessary, however, to prevent re-appearance of tumors.

An experimental animal (horse) has received sub-cutaneous injections of minced tumor from a total of seven patients. White blood cells separated from the horse's blood has been injected into tumors of patients. No consistent effects on tumors so injected have been observed.

Basal Cell Carcinoma: Patients with extensive disease have been treated with Methotrexate given systemically. Tumors have responded with a hemorrhagic reaction and a few tumors have clinically disappeared. Combined use of local X-ray and Methotrexate has suggested that these agents may act synergistically if given alternately at one week intervals.

The dependence of basal cell tumors on connective tissue stroma has been confirmed. Clinical observations in patients has supported observations made by others working with animals in experimental carcinogenesis, namely, that carcinogenesis is more dependent upon changes in the dermis than on alterations in the epidermis. The supporting clinical observation has been the appearance of numerous basal cell tumors in skin (donor site, thigh) grafted to the forehead.

Psoriasis: Recognition of psoriasis as a more systemic disease, rather than one confined to the epidermis, has become more apparent through the identification of a characteristic arthritis. Both the skin lesions and the arthritis have been observed to be altered beneficially by Methotrexate given systemically. Currently, a joint

project of the Dermatology Service, NCI and the Arthritis and Rheumatism Branch, NIAMD is being pursued to test the validity of this observation. This is a "double blind" study, the results of which will not be ascertained for several months.

The suspicion that the epidermis is not the prime site of the disease has been supported by several manipulative procedures on the skin. For example, damage of the sub-epidermal connective tissue naturally (such as in linea distensae) or experimentally (such as by deep electro-desiccation or by surgically undermining the epidermis) is associated with the absence of or clearing of the psoriatic epidermal lesion.

Elevation of serum uric acid has been found to be characteristic of psoriasis. Patients with the disease have been found to over-incorporate C-14 glycine into uric acid. Although this may be secondary to increased mitotic activity of the hyperplastic psoriatic epidermis, it may signify some other abnormality and may relate to the occurrence of arthritis in psoriasis.

Significance to Cancer Research:

Project directly concerned with study of, and therapeutic influences on, cancer and related conditions.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Van Scott, E. J., Shaw, R. K., Crounse, R. G. and Condit, P. T., Effects of Methotrexate on Basal Cell Carcinomas, A.M.A. Arch. Dermat. (in press).

2. Utz, J. P., Van Scott, E. J., Benton, H. W., Edgcomb, J. H., Bunim, J. J., Bell, N. H., and Kaufman, H. E., Sarcoidosis: Clinical Staff Conference at the National Institutes of Health, Ann. Int. Med. 51: 1356-1370, 1959.

Serial No. C-7-728
1. General Medicine Branch
2. Dermatology Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Structural and Physiological Characteristics of Normal and Pathological Epidermis and Related Epithelial Tissues.

Principal Investigator: E. J. Van Scott, M. D.

Other Investigators: Richard P. Reinertson, M. D.

Cooperating Units: Metabolism Service, NCI, to some degree.

Man Years: (calendar year 1960) Patient Days (calendar year 1960):

Total:	3
Professional:	1
Other:	2

Project Description: (Continuation of C7-728(c) of 1959

Objectives: To investigate structural and physiological characteristics in epidermal and supporting tissues in pursuit of understanding wide ranges of growth activity of epidermal tissues and regulatory factors thereof.

Methods Employed:

Tissues are studied histologically and from reconstructions of specific structures. Total number of cells and mitotic figures counted microscopically, volume of specific tissue structures calculated from microscopic measurements, geometric relationships determined. Further comparisons are made on the basis of structural changes following manipulative procedures and following drugs.

Split-thickness specimens of normal and pathological epidermis are tissue cultured under varying conditions of pH, CO_2 tension, and O_2 tension to study influences on keratinization.

Part B included: Yes /X/ No / /

Patient Material:

Tissues studied or used are from hospitalized patients and outpatients of Dermatology Service, NCI.

Major Findings:

Although the range of responses that may be pursued by epithelial cells appears to be a property of the cell line, the specific response that a cell undergoes is determined by the environment in the particular connective tissue stroma. Preliminary observations that lead to this conclusion earlier have been confirmed and extended. Examples of these observations are as follows:

A. Split thickness specimens of buccal mucous membrane (mucosa + sub-mucosal connective tissues) transplanted to the skin survive and retain histologic characteristics of mucous membrane.

   1. Pure mucosa (devoid of connective tissue) transplanted to skin.

      (a) keratinizes when implanted sub-epidermally.
      (b) forms duct-like structures when implanted in the lower corium.

B. Scalp hair roots with intact native connective tissue when transplanted to the skin of the back retain normal structural architecture.

   1. Pure external hair roots sheaths (devoid of connective tissue) transplanted to the skin of the back form duct-like structures when implanted in the lower corium.

C. Basal cell carcinoma with stromal connective tissue intact transplanted into the corium or sub-cutaneous tissue survives and remains unaltered.

   1. Pure basal cell carcinoma (devoid of stromal connective tissue) transplanted to

      (a) lower corium degenerates, keratinizes, or forms duct-like structures.

      (b) when transplanted sub-cutaneously the tumor tissue degenerates.

Study of factors influencing keratinization of epidermal cells in tissue culture has indicated that proliferation and keratinization of epidermal cells are enhanced by an atmosphere of high CO_2 tension. Similar enhancement is observed when the culture fluid medium has a pH above normal. Effects of chemotherapeutic agents on epithelial cells have been further studied and identified. Those following Methotrexate have been more fully studied and are characterized by reversible suppression of mitoses. Therapeutic doses of the drug may cause complete absence of mitoses in the hair root, epidermis, and duodenal mucosa. In the hair, cells of the lower matrix are more sensitive to the drug in that mitoses may be completely absent here, whereas they are present in the upper matrix. Associated with suppression of mitoses is the appearance of microscopically visible bodies in the cytoplasm. The nature of these is unknown. Similar bodies have been noticed in the cells of the duodenal mucosa following Methotrexate. These are being studied by Dr. Jerry Trier, Metabolism Service, NCI, by both microscopic and electron microscopic techniques.

Significance to Cancer Research:

The studies are directly concerned with cellular reproduction, destruction, and quiescence. The varying responses of epithelial cells to environmental manipulations provide possible information regarding characteristic responses of these cells in malignant transformation.

Proposed Course of Project:

Continuation along lines generally as indicated in the foregoing.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Crounse, R. G. and Van Scott, E. J., Changes in Scalp Hair Roots as a Measure of Toxicity from Cancer Chemotherapeutic Drugs. J. Invest. Dermat. 35: 83-90, 1960.

2. Van Scott, E. J. and Reinertson, R. P., The Modulating Influence of Stroma on Cells Derived from the Epidermis Studied in Human Autotransplants. J. Invest. Dermat. (in press).

Serial No. C-7-730
1. General Medicine Branch
2. Dermatology Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: The Biochemistry of Normal and Pathological Epidermis.

Principal Investigator: Simon Rothberg, Ph.D.

Other Investigators: Eugene J. Van Scott, M. D.

Cooperating Units: None

Man Years (calendar year 1960): Patient Days (calendar year 1960)

Total: 2
Professional: 1
Other: 1

Project Description: Continuation of C7-730 of 1959

Objectives: To study the biosynthesis of the major proteins in normal and abnormal epidermis. Knowledge of this process will aid in comparisons of the proteins synthesized in normal and diseased states.

To study the time sequence of biosynthesis and fibrillar aggragation of protein in epidermal tissue.

To study in epidermal tissue the rate of ascent of labelled protein from the site of synthesis to the epidermal surface.

To evaluate the differences between the major keratinous proteins of normal and pathological human epidermis in order to determine whether abnormal keratins may exist.

To study the biosynthesis of desoxyribonucleic acid (DNA) in epidermal tissues by incorporation of thymidine-H^3 into DNA.

Part B included: Yes /X/ No / /

Methods Employed:

The glycine-C^{14} incorporated into normal human epidermal tissue is obtained from stratum corneum scraped from the backs of patients receiving the radioactive amino acid. The amino acids and peptides are separated by dialysis from the proteins; the proteins in turn are separated in phosphate buffer (pH 7.3) into the soluble fraction and the insoluble residue. The salt free protein is acid hydrolysed and glycine separated from the other amino acids by a combination of high voltage electrophoresis and partition chromatography. The specific activity of the purified glycine is determined using a windowless gas flow system.

Scales of psoriatic patients are collected daily and are treated in a similar fashion in order to isolate the glycine and determine its specific activity.

Further separation of the proteins present in the soluble and insoluble fractions and the subsequent isolation of the glycine in each of these proteins relate the proteins in a time sequence of biosynthesis.

These related proteins are also characterized by the patterns of peptide composition of the individual proteins. Single enzyme (tryspin or chymotryspin) hydrolysis of the protein and separation of the peptides obtained by improved methods using high voltage electrophoresis are used.

The rate of ascent in the human epidermis of a labelled amino acid incorporated into the proteins is followed by radioautographs of serial biopsy specimens. The biopsy material is fixed and then vertically cut into 8 micron sections. These sections are placed next to nuclear tract plates for timed exposures (many months).

Thymidine H^3 added the media in a tissue culture of epidermal tissue is incorporated only into DNA. The presence in the isolated DNA of radioactivity thus reflects growth of the tissue.

Rabbit epidermis isolated from the total skin of the animal is examined for arginase activity.

Major Findings:

For normal epidermal tissue the turnover time, as indicated from the time of introduction of the radioactive glycine until the time of its

presence in the outermost layers of the stratum corneum, was found to be approximately 28 days. On the other hand the turnover time as found from analysis of the radioactive scales of psoriatics was found to be between 3-5 days. Whether this difference is due to an increased mitotic index or to acceleration of the rate of protein synthesis in the cells of the epidermal tissue is as yet unclear.

Preliminary data on the different proteins isolated from the epidermis seem to indicate similar levels of activity for synthesis at similar times except for a fraction isolated at pH 4.5, which seems to precede the other proteins in its peak activity.

Arginase was found to be present in rabbit epidermis. This finding was expected and discounts a published report that arginase is induced in rabbit skin by the Shope papilloma virus.

Significance to Cancer Research:

The stimulants of increased growth can be further studied when the exact nature of the growth pattern is clear. In the case of the increased mass of protein found in the pathological stratum corneum of psoriatics, it is not clear whether accelerated mititoc division or rapid protein synthesis within the individual cell is responsible for the increased protein mass found. The studies reported here are the initial steps to aid in describing the meaning of increased epidermal growth.

Proposed Course:

Studies of growth in normal and abnormal epidermis will continue with experiments of protein synthesis both in vivo and in tissue culture. DNA synthesis in tissue culture will be studied to determine if the components of an abnormal epidermis in vivo act in a similar fashion in tissue culture.

The keratinous fractions of normal and abnormal epidermis will be further purified, their physical properties and amino acid structure determined, in order to clearly indicate whether an abnormal protein is synthesized by the abnormal epidermis.

Serial No. C-7-730

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Crounse, R. G. and Rothberg, S., The distribution of arginase in human hair roots. J. Invest. Dermat. 35, 107, (1960).

2. Crounse, R. G. and Rothberg, S., Evaluation of the enzymes of the Krebs-Henseleit urea cycle in human epidermis. J. Invest. Dermat. (in press).

3. Crounse, R. G. and Rothberg, S., Enzymes of the Krebs-Henseleit cycle in human epidermis. Arch of Biochem. and Biophys, 91, 151 (1960).

4. Rothberg, S., and Van Scott, E. J., Localization of arginase in rabbit skin. Nature (submitted for publication).

Serial No. NCI-6-702-(c)
1. General Medicine Branch
2. Metabolism Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Serum Proteins Studies

Principal Investigator: John L. Fahey, M.D.

Other Investigators: None

Cooperating Units: For the study of neoplasms in mice - Laboratory of Biology, NCI (Dr. M. Potter) and the National Institute for Medical Research, London, (Dr. Askonas).

Man Years (calendar year 1960):

Total	2
Professional:	1
Other	1

Project Description:

Objectives: The object of this project is to determine physico-chemical, metabolic and other characteristics of the serum proteins found normally and in association with malignant disease, the role of selected proteins in the modification or manifestations of disease, and the usefulness of serum proteins as easily accessible indices of disease activity or therapeutic effectiveness.

Methods Employed: Quantitative and qualitative chemical, physical, and immunochemical procedures, as well as tests for biologic activity are used to measure and characterize, where possible, individual proteins. Radioisotopically labelled (I^{131} or C^{14}) proteins in tracer amounts are utilized for metabolic studies. Fractionation of the complex mixture of normal serum proteins is done by electrophoretic, chromatographic, solubility and other procedures.

Patient Material: Sera were obtained from patients admitted to the Metabolism Service, NCI, from cooperating units throughout the National Cancer Institute and the National Institutes of Health and also from physicians in the metropolitan Washington area and throughout the country. Clinical information was available on all

Part B included Yes X No

of these patients and selected patients were admitted to the Clinical Center for detailed study.

Major Findings: Observations on the physicochemical variety of antibodies were extended. It was possible to show that some antibodies were solely among the 6.6S gamma globulins, some were 18S gamma globulins, and certain antibody activities were present in both the 6.6S and 18S groups of gamma globulins. Anti-thyroglobulin antibodies of the 6.6S type were found in sera from five patients while two had appreciable amounts of 18S antibodies as well. In sera from patients with lupus erythematosus most of the antibodies against liver nucleoprotein were shown to be 18S antibodies whereas serum antibodies reacting with thymus nucleoprotein were only among the 6.6S gamma globulins. The LE factors were present only among the 6.6S gamma globulins. The techniques used in the studies will be of value in further investigations of the nature of antibodies.

Studies of the proteins formed in malignant plasma-cells were facilitated by use of transplantable mouse plasma-cell tumors. The myeloma proteins formed in each of 8 tumors were found to have distinctive properties that remained unchanged through many transplant generations. These distinctive properties were reflected in the electrophoretic mobility, the size and ultracentrifugal behavior, the hexose content and immunoelectrophoretic properties of the myeloma proteins.

Significance to Cancer Research: The individuality of the myeloma proteins and malignant plasma-cells tumors indicate the functional distinctness of each tumor. These observations support the possibility that each neoplasm arose from a malignant transformation in a single plasma-cell clone.

Proposed Course of the Project: Detailed study of the proteins formed in normal and malignant plasma-cell are to be extended by enzymatic fragmentation of purified molecules. Physicochemical, immunochemical and metabolic appraisal of the protein parts is anticipated.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Fahey, J.L.: Separation of Serum Antibody Activities by Anion-Exchange Cellulose Chromatography. Science, 131: 500-501, 1960.

2. Fahey, J.L. and Goodman, H.C.: Characterization of Anti-Thyroglobulin Factors in Human Serum. J. Clin. Invest., 39: 1259-1265, 1960.

3. Goodman, H.C., Fahey, J.L. and Malmgren, R.A.: Serum Factors in Lupus Erythematosus and Other Diseases Reacting with Cell Nuclei and Nucleoprotein Extracts: Electrophoretic, Ultracentrifugal and Chromatographic Studies. J. Clin. Invest., 39: 1595-1605 , 1960.

4. Fahey, J. L.: Anion-exchange Cellulose Fractionation of Serum Proteins, in Proteids of Biological Fluids. 8th Colloquium, in press,(1960).

5. Fahey, J.L., Dutcher, T.E. and Goodman, H.C.: Cell Nuclei and Gamma Macroglobulins. Proc. Roy. Soc. of Med. Proc. Roy. Soc. of Med. 53: 627-630, 1960.

6. Fahey, J.L., Potter, M. and Nathans, D.: Myeloma Proteins and Macroglobulins Associated with Plasma Cell Tumors in Experimental Animals. Acta Union Internationale Contre Le Cancer, 16:1136-1140, 1960.

7. Fahey, J.L. and Askonas, B.A.: The Significance of Myeloma Proteins as Products of Plasma-Cell Metabolism, in Proteids of Biological Fluids, 8th Colloquium, in press,(1960).

Serial No. NCI-6-704(C)
1. General Medicine Branch
2. Metabolism Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies of Porphyrin Metabolism in the Tumor Bearing Host and Porphyria.

Principal Investigator: Donald P. Tschudy

Other Investigators: Annie Collins, Emmanuel Hellman, James Rose

Cooperating Units: Laboratory of Pathology, NCI; (Dr. Clyde Dawe)

Man Years (calendar year 1960)
Total: 2 1/2
Professional: 1 1/2
Other: 1

Project Description:
Objectives: To study tumor host interrelationships from the standpoint of porphyrin synthesis in liver and red cells.

To study the effect of diet, vitamins, drugs and hormones on porphyrin biosynthesis

To study biochemical abnormalities of acute intermittent porphyria.

Methods employed:
A. Quantitative enzyme determinations.
B. Assay of urine for porphyrins, porphyrin precursors, uric acid and amino acids.
C. Use of tissue slices and homogenates in studying Kreb's cycle and lipid synthesis.

Patient Material: Patients with malignancies and acute hepatic porphyria.

Major Findings: The previously described decreased in hepatic ALA dehydrase in tumor bearing hosts has been seen without exception in all animals bearing transplanted or spontaneous tumors which have attained significant size. Virus induced tumors appear to constitute

Part B included Yes X No____

an exception to this finding, in that the livers of animals bearing massive tumors (of various types) induced by polyoma virus have not shown a decrease of hepatic ALA dehydrase. Whether the failure of ALA dehydrase to decrease in the livers of hosts bearing polyoma virus induced tumors is a specific biochemical difference between virus tumors and those previously studied, or whether the difference is related to the degree of autonomy of the tumor is unknown.

The porphobilinogenuria of experimental porphyria in rats can be completely prevented by administration of adequate amounts of diet. The active components are protein and carbohydrate.

In several patients with acute hepatic porphyria, a syndrome of hypo-osmolarity with urinary salt wasting has been described and investigated. This is thought to be related to inappropriate persistent ADH secretion secondary to the CNS lesions of porphyria.

Significance to Cancer Research: The decrease in hepatic ALA dehydrase in tumor bearing hosts may be related to the etiology or degree of autonomy of a tumor.

Proposed Course of the Project:

A. To continue studies of the mechanism of decreased hepatic ALA dehydrase in tumor bearing hosts.

B. To continue studies of biochemical abnormalities of acute porphyria in man and experimental porphyria in animals.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publication

Publications other than abstracts:

1. Tschudy, D.P., Collins, A., Caughey, W.S. and Kleinspehn, G.G.: Inhibition of δ-Aminolevulinic Acid Dehydrase by δ-Oximinolevulinic Acid. Science, 131: 1380-1381, 1960.

Serial No. NCI-6-721 (C)
1. General Medicine Branch
2. Metabolism Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Metabolism of Nutrients and Calories in Neoplastic Disease.

Principal Investigator: Donald M. Watkin, M.D.

Other Investigators:

Cooperating Units:

Man Years: (calendar year 1960)
Total: 4.0
Professional: 4.0
Other:

Project Description:

Objectives: Investigation of the over-all and intermediary metabolism of fat, carbohydrate, protein, minerals, electroytes, vitamins, calories and water in patients during the natural course of neoplastic disease, and during their responses to nutritional, physiological, pharmacological, endocrine, surgical or radiologic stress.

Major Findings: The principal investigator resigned as of June 30, 1960. This project will now be incorporated into project NCI-6-727 (C).

Part B included Yes___ No X

Serial No. NCI-6-723 (C)
1. General Medicine Branch
2. Metabolism Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A:

Project Title: Studies of Human Serum Albumin and Gamma Globulin Labeled with I^{131}.

Principal Investigator: Thomas A. Waldmann, M.D.

Other Investigators: Robert Gordon, Jr., Thomas Dutcher, John L. Fahey.

Cooperating Units: Laboratory of Pathology, National Cancer Institute, Laboratory of Cellular Physiology and Metabolism.

Man Years (calendar year 1960)
Total: 1
Professional: 1
Other: 0

Project Description:

Objectives: To determine the pathogenesis of the serum protein abnormalities in patients with lymphoma, gastrointestinal neoplasm, idiopathic hypoalbuminemia, agammaglobulinemia and dysgammaglobulinemia and to develop quantitative techniques for the detection of protein loss into the gastrointestinal tract.

Methods Employed: Normal human albumin and gamma globulin labeled with radioiodine were given intravenously to individual receiving Lugol's solution. Blood, urine, and stool were assayed for radioactivity for three weeks following administration of the labeled protein and the protein pool size, life span, and rate of synthesis were determined.

Polyvinylpyrrolidone (PVP) labeled with radioiodine or albumin labeled with radiochromium were administered intravenously and the subsequent four days of stool were collected and assayed for radioactivity to detect gastrointestinal protein loss.

Part B included Yes___ No_X_

Patient Material: Protein turnover studies have been performed on groups of patients with idiopathic hypoalbuminemia, agammaglobulinemia, macroglobulinemia, cryoglobulinemia, and lymphoma.

Major Findings: A new method for the detection of protein loss into the gastrointestinal tract utilizing radio chromium labeled albumin has been developed. Following intravenous administration of the chromium labeled albumin to controls less than 0.25 percent of the administered radioisotope appeared in the subsequent four day stool collection. In patients with known protein leaking syndromes, 5 to 22 percent of the administered activity appeared in the stool collection.

On the basis of the I^{131} Albumin and Cr^{51} Albumin and I^{131} PVP studies, the 23 patients with idiopathic hypoalbuminemia and edema could be differentiated into two major groups, the hypoanabolic and hypercatabolic. Two patients with hypoanabolic hypoproteinemia had an almost complete defect in albumin synthesis. There was also a marked prolongation of albumin survival, indicating that there was an associated defect in albumin degradation in these patients.

The remaining patients with hypercatabolic hypoproteinemia have been shown to have a normal or slightly increased albumin synthesis, but marked gastrointestinal protein loss. Four patients had gastrointestinal protein loss in association with constrictive pericarditis. Following pericardectomy in these patients, the protein concentration and survival returned to normal.

In twelve of the remaining patients, the protein loss has been shown to be related to a previously undescribed entity with dilated lymphatic channels of the small intestinal mucosa and mesentery.

Gastrointestinal protein loss has been demonstrated as a significant factor in some patients with sprue, agammaglobulinemia, hypertrophic gastric rugae, and acute idiopathic hypercatabolic hypoproteinemia.

There was a significant reduction in the albumin synthetic rate in all 15 patients with lymphoma and hypoalbuminemia studied. The albumin survival was prolonged in the 2 patients with lymphoma and large effusions, while in the remaining patients the survival was shorter than normal, or at the lower limit of normal. Albumin loss into the gastrointestinal tract has not been demonstrated in any of these patients.

The survival of I^{131} gamma globulin has been shown to be prolonged in patients with non productive agammaglobulinemia, and shortened in patients with gastrointestinal protein loss, and in patients with macroglobulinemia.

Significance to Cancer Research: Patients with neoplasma frequently have abnormalities in the concentration and nature of the serum proteins. These studies are directed toward understanding the factors controlling the synthesis, degradation, and loss of these proteins.

Proposed Course: To continue studies directed toward understanding the pathogenesis of the serum protein abnormalities associated with neoplastic diseases and idiopathic hypoproteinemia.

Serial No. NCI-6-725 (C)
1. General Medicine Branch
2. Metabolism Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Vitamin B_{12} metabolism in normal subjects and patients with neoplastic and other diseases.

Principal Investigator: Donald M. Watkin, M.D.

Other Investigators: None

Cooperating Units: None

Man Years
Total: 1
Professional: 1
Other:

Project Description:

Objectives: Quantitative observations on the metabolism of vitamin B_{12} in man including its absorption from the gastrointestinal tract, its distribution in the organs of the body, its level in plasma, its binding to plasma proteins and its excretion by the kidney in normal subjects and those with neoplastic disease.

Major Findings: On June 30, 1960 this project was terminated by the resignation of the principal investigator.

Part B included Yes___ No X

Serial No. NCI-6-726 (C)
1. General Medicine Branch
2. Metabolism Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Renal clearance studies in patients with neoplastic disease.

Principal Investigator: Donald M. Watkin, M.D.

Other Investigators:

Cooperating Units: None

Man Years (calendar year 1960)
Total: 1/24
Professional: 1/48
Other: 1/48

Project Description:

Objectives: To estimate the glomerular filtration rate, effective renal plasma flow and tubular mass and to investigate the mechanisms by which the kidneys handle metabolites such as uric acid and calcium in patients with neoplastic disease. To ascertain the kidneys role in such disorders as hyperuricemia and hypercalcemia when these accompany neoplastic disease.

Major Findings: On June 30, 1960, this project was terminated by the resignation of the principal investigator.

Part B included Yes___ No X

Serial No. NCI-6-727 (C)
1. General Medicine Branch
2. Metabolism Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Gross Body Composition

Principal Investigator: N.I. Berlin, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960)
Total: 6
Professional: 6
Other: o

Project Description:

Objectives: This project is designed to study the changes in gross body composition during the course of neoplastic diseases, and to correlate these changes with the clinical course of the patient and other laboratory data.

Methods Employed: Gross body composition will be determined from the body density and total body water. Body density will be determined from body mass and body volume, as measured by the Siri body volume apparatus; body water will be determined with tritiated water.
Metabolic balance of N,K,P,Ca,Na and
Newburgh insensible water loss technique for calculation of caloric expenditure.

Patient Material: 9 patients with various neoplasms, 2 obese subjects with Dr. Buskirk, NIAMD and 1 patient with refractory anemia.

Major Findings:
Gross Body Composition:

Analysis of the data obtained from the three obese subjects and from 6 additional subjects indicates that the measurement of caloric expenditure in man is feasible, but requires approximately 30 days of observation on a metabolic balance regime during which time it must be assumed that caloric expenditure is constant.

Part B included Yes___ No X

Caloric expenditure is calculated from: Caloric intake - caloric content feces and urine + 9.0 Δ fat (gr/day). Where Δ fat is rate of change of body fat content in grams/day, Δ fat is determined from body density and total body water measurements. Balance of lean tissue is principally determined from N balance data.

As measured in this manner caloric expenditure is in good agreement with that estimated by the Newburgh insensible water loss technique in less than 1/2 of the subjects studied. There does not appear to be some common factor in those patients in which the 2 methods do not agree.

Anemia of Cancer: The section on Anemia of Cancer of this project has been transferred to NCI-6-738 (C).

Significance to Cancer Research: This project is part of the studies carried out in the area of the tumor-host relationship. During the course of a neoplastic disease, large changes in body weight occur, particularly weight loss. These must represent some changes brought about in the metabolism of the host. As a preliminary and a necessary adjunct to the study of these changes in the host metabolism, it is necessary to know the changes occurring in gross body composition.

Proposed Course: Two types of patients will be studied by these methods. The first will consist of individuals in the rapid weight loss phase of their neoplastic disease, and the second individuals early in the course of the disease, who will be available for repeated follow-up studies. The long range future will depend upon the nature of the weight loss. At present it can be speculated that fat loss is a major component, in which case the factors controlling fat metabolism will be studied.

Serial No. NCI-6-729 (C)
1. General Medicine Branch
2. Metabolism Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies of Nitrogen Metabolism using N^{15}.

Principal Investigator: Donald P. Tschudy

Other Investigators: Emmanuel Hellman

Cooperating Units: Laboratory of Physiology

Man Years (calendar year 1960)
Total: 3/4
Professional: 0
Other: 3/4

Project Description:

Objectives:

A. Through the use of mathematical models of nitrogen metabolism to study the effects of such variables as diet, hormones, tumor growth and radiation on the kinectics of body nitrogen.

Methods Employed:

A. Synthesis of N^{15} labelled amino acids.
B. Isolation of urea and ammonia from urine
C. Preparation of materials for mass spectrometry
D. Metabolic balance studies.

Patient Material: A patient with lymphosarcoma has been studied through most of the course of her disease. Several other patients with malignancies have been studied before and after radiation therapy.

Major Findings: The data from this year are just becoming available and have not yet been analyzed.

Significance to Cancer Research: These methods permit the quantitative study of the tumor bearing host with regard to nitrogen metabolism and the effects of many variables such as diet, therapy, etc. thereon.

Proposed course of the Project: To study the effects of radiation, steroids and tumor growth more extensively.

Part B included Yes X No___

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publication

Publications other than abstracts:

1. Tschudy, D.P., Weissman, S.M., Baccus, H. and Eubanks, M.: The Use of Precursor Product Relationship in Determining Half Life of Human Serum Albumin. J. Lab. and Clin, Med. (in press) 1960.

Serial No. NCI-6-732 (C)

1. Beneral Medicine Branch
2. Metabolism Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Investigation of Malignant Reticular Disease.

Principal Investigators: John L. Fahey, M.D.

Other Investigators: None

Cooperating Units: In specific aspects of the work:
Pathologic Anatomy Branch, NCI (Dr. Dutcher)
Institute for Allergy and Infectious Diseases
(Dr. J. Utz)
Chemotherapy Service, NCI (Dr. Frei)

Man Years (calendar year 1960)

Total:	1 1/2
Professional:	1
Other:	1/2

Project Description:

Objectives: The clinical and biochemical characteristics of malignant reticular and related diseases are to be explored with particular attention to diseases with associated macroglobulinemia, cryoglobulinemia or other serum protein abnormality. It is hoped to gain insight into the mechanisms responsible for the major disabling features of these illnesses, to clarify diagnosis and prognosis, and to improve treatment.

Methods Employed: Chemical and physicochemical technics, including electrophoresis, ultracentrifugation and chromatography are employed for quantitative and qualitative assessment of serum protein changes. Quantitative immunologic techniques are employed for assessment of serum antibody levels and of response to antigenic stimulation. Histologic evaluation is conducted by histochemical, immunohistologic, electron microscopic and standard morphologic procedures. Clinical evaluation is based on specific protocols derived from previous experience.

Part B included Yes X No

Major Findings: Patients with Waldenström's macroglobulinemia have markedly elevated serum viscosity. Evidence that the macroglobulins were responsible for the increased viscosity, retinal vessel dilitation and hemmorrhage and other disease manifestation was obtained by reduction of the macroglobulin level by plasmapheresis. Marked retinal changes were successfully treated by this procedure. Plasmapheresis proved to be a safe and feasible means of reducing elevated serum macroglobulin levels.

Further evidence was obtained that the lymphocytoid plasma-cells found in Waldenstrom's macroglobulinemia were the source of the serum macroglobulin. By employing specific fluorescent-labeled antisera against 18S gamma globulin, the lymphocytoid plasma-cells were shown to contain 18S macroglobulin, strong presumptive evidence that these cells are the site of macroglobulin synthesis.

The serum protein changes in acute and chronic leukemias were investigated by quantitative paper electrophoresis. Myelogenous and lymphocytic leukemia differed in their effects on the alpha and gamma globulins. The gamma globulins typically were increased in myelogenous leukemia and decreased or unchanged in the lymphocytic leukemias. Investigations of antibody responses indicated that the response to antigen stimulation was impaired and the isohemagglutinin levels were reduced in most cases of chronic lymphocytic leukemia. Most but not all patients with impaired immune status also had low gamma globulin levels. These changes were not regularly encountered, however, in the acute leukemias.

Significance to Cancer Research: Progressive clinical deterioration due to high serum viscosity in Waldenström's macroglobulinemia may be halted and even reversed by plasmapheresis. This has proved to be critical to the survival of two patients with rapidly advancing disease. Plasmapheresis constitutes an important therapeutic procedure for this disease.

Identification of macroglobulin-producing cells has been shown to be feasible by immunofluorescent techniques, and investigation of the distribution of these cells has begun.

Gamma globulin and immune changes in lymphocytic malignancy were shown to make the patient more susceptible to severe and dangerous infections.

Proposed Course of the Project: Therapeutic observations in macroglobulinemic diseases will continue and similar observations in cryoglobulinemic diseases will be undertaken. The effects of malignant and nonmalignant diseases on normal gamma globulin components and immune mechanisms will be explored. An effort will be made to define the criteria by which nonmalignant diseases with associated cryoglobulins or increased gamma macroglobulins may be distinguished from malignant diseases with similar serum protein changes.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts:

1. Schwab, P.J. and Fahey, J.L.: Treatment of Waldenström's Macroglobulinemia by Plasmapheresis. New Eng. J. Med., 263: 574-579, 1960.

2. Schwab, P.J., Okum, E. and Fahey, J.L.: Reversal of Retinopathy in Waldenström's Macroglobulinemia by Plasmapheresis. A.M.A. Arch. Ophthalmology, 64: 515-521, 1960.

3. Dutcher, T.F. and Fahey, J.L.: Immunocytochemical Demonstration of Intranuclear Localization of 18S Gamma Macroglobulin in Macroglobulinemia of Waldenström. Proc. Soc. Exp. Biol. and Med., 103: 452-455, 1960.

4. Fahey, J.L. and Boggs, D.R.: Serum Protein Changes in Malignant Diseases. I. I. The Acute Leukemias. Blood, 16: 1479-1490, 1960.

5. Boggs, D.R. and Fahey, J.L.: Serum Protein Changes in Malignant Diseases II. The Chronic Leukemias, Hodgkin's Disease and Malignant Melanoma. J. National Cancer Inst. 1960, (in press).

6. Silver, R.J., Utz, J.P., Fahey, J.L. and Frei, E.: Antibody Response in Patients with Acute Leukemia: Its Nature and its Relation to Bacterial Infection. J. Lab. and Clin. Med. 1960, (in press).

7. Shaw, R.K., Boggs, D.R., Szwed, C., Fahey, J.L., Frei, E., Morrison, E. and Utz, J.P.: Infection and Immunity in Chronic Lymphocytic Leukemia. A.M.A. Anch. Int. Med., 106: 467-478, 1960.

Serial No. NCI-6-738 (C)
1. General Medicine Branch
2. Metabolism Service
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: A Study of the Factors Controlling Red Blood Cell Synthesis and Destruction

Principal Investigator: Thomas A. Waldmann, M.D.

Other Investigators: Nathaniel I. Berlin, M.D., Edgar Levin, M.D. and Wendell F. Rosse, M.D.

Cooperating Units: None

Man Years (calendar year 1960)
Total: 1
Professional: 1
Other: 0

Project Description:

Objectives: To study the factors controlling red blood cell synthesis and destruction in normal and in pathological states.

Methods Employed: Quantitation of erythropoiesis by the use of Cr^{51} labeled red cells to determine the total circulating red cell volume, Fe^{59} labeled transferrin to determine the site and rate of red cell production and glycine-2-C^{14} to determine the red cell life span. Assay of erythropoiesis stimulating activity in the fasted rat and mouse by the red cell radio-iron uptake and reticulocytosis.

Patient Material: Patients with polycythemia secondary to cerebellar hemangioblastoma, hypernephroma, pheochromocytoma and adrenal cortical tumors, patients with other neoplastic diseases and refractory anemia.

Major Findings: The total red cell volume and rate of red cell synthesis was increased in the patients with a cerebellar hemangioblastoma, hypernephroma or adrenal cortical tumor. Non-dialyzable factors with marked erythropoiesis stimulating activity were isolated from the fluid from the cystic cerebellar hemangioblastoma and from the plasma an and tumor homogenate of the patient with the pheochromocytoma. These data support the concept that the polycythemia associated with cerebellar hemangioblastomas and pheochromocytomas is caused by the production of a red cell stimulating factor by the tumor.

Part B included Yes X No___

With radioactive iron it was demonstrated that 20 hours after administration of methotrexate complete inhibition of erythropoiesis occurs. This inhibition persists for approximately 3 days - with apparent complete recovery. Surface counting of the isotope shows that the iron is diverted principally to the liver - and that when erythropoiesis returns this iron is available for hemoglobin synthesis.

A study of 8 patients with refractory anemia shows that all had a low total red cell volume - with a tendency for a normal plasma volume. The rate of disappearance of radio iron from the plasma was increased in 1, normal in 2, and decreased in 5. The uptake of radio iron by red cells was low in all instances. Cr^{51} red cell survival curves, while difficult to interpret suggest an element of hemolysis in all patients. Autopsy in 7 patients shows hemosiderosis of the liver in all instances. Erythropoietin was present in urine and plasma of the 3 patients of the group studied. Of the 2 patients treated with testosterone a limited effect of doubtful clinical value was obtained in 1 patient.

Preliminary studies utilizing parabiotic rats indicate that anoxia of the kidney is necessary for the accelerated production of red cells in response to hypoxia and tend to confirm the suggestion that the kidney is the major site of erythropoietin production.

The liver has been previously suggested as a major site of erythropoietin degradation. Through the use of dogs in whom all the venous blood is shunted through the liver before returning to the heart it has been shown that erythropoietin is not degraded by one passage through the liver. Following cerebellectomy in the dog there is no reduction in the total red cell volume even though certain tumors of this area produce polycythemia.

Following administration of dessicated thyroid to the dog there was a 25 percent increase in the total red cell volume and rate of red cell synthesis but no change in the red cell life span.

Significance to Cancer Research: Polycythemia is associated with certain malignant diseases. These studies are directed toward understanding this association. Anemia is a frequent complication in the neoplastic diseases. A study of the factors controlling erythropoiesis is necessary for an understanding of the mechanisms involved in the production of anemia - in patients with cancer.

Proposed Course of Study: To continue the study of the factors controlling erythropoiesis in the experimental animal and man. To conduct studies directed toward determining the chemical nature and mode of action of the erythropoiesis stimulating factor so produced by tumors.

PHS-NCI
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publication

Publications other than abstracts from this project:

1. Weissman, S.M., Waldmann, T.A., Berlin, N.I.: Quantitative Measurement of Erythropoiesis in the Dog. Am. J. Physiol. 198: 183-186, 1960.

2. Waldmann, T.A., Weissman, S.M., Berlin, N.I.: The Effect of Splenectomy of Erythropoiesis in the Dog., Blood, 15: 873-883, 1960.

3. Waldmann, T.A., Levin, E., Baldwin, M.: The Association of Polycythemia with a Cerebellar Hemangioblastoma: The Production of an Erythropoiesis Stimulating Factor by the Tumor. Amer. J. Med. (in press) 1960.

4. Condit, P.T., Berlin, N.I. and Nathan, D.G.: Studies on the Folic Acid Vitamins, VI. The Effect of Amethopterin on Erythropoiesis in Man. Cancer, 13: 245-249, 1960.

Serial No. NCI-688
1. Radiation Branch
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Development, Fabrication and Maintenance of Specialized Equipment for Radiation Research

Principal Investigator:

Calvin R. Mencken

Other Investigators:

Edward J. LeBrun and Ralph C. Whittaker

Cooperating Units:

None

Man Years (calendar year 1960)
Professional: 2
Other : 1
Total: 3

Project Description:

Objectives: To design and fabricate laboratory instruments and devices that are not available on the open market, and/or incorporate refinements to said devices to make them applicable for the unusual services that are required in radiation research. To service or assist the investigators in maintaining such equipment as the Van de Graaff generators and fabricate special parts for same, as requested by the investigators. To service other equipment as requested. The accompanying list of projects is condensed and does not give a complete or wholly accurate description of every task involved. The more significant ones will be designated by an asterisk (*). If more complete and accurate information is desired, it is suggested that the investigator whose name accompanys the item be consulted.

It will be noted that a part of the list is involving Glass Blowing. This will be listed separately and will be designated as such.

Project Description: (Continued)

Major Projects: Radiation Branch

Dr. Louis Avioli:
Design and fabricate constant infusion pump.

Glass Blowing:
* Design pH cup.
Fabricate self-filling dispenser.
Fabricate manifold.

Dr. Kirkland Brace:
Fabricate treatment wedges as per specifications.
Fabricate plastic and metal for X-ray locations.
Fabricate support on Lumicon to hold X-ray cassettes.
Fabricate plastic display case for human skull.

Dr. Falconer Smith:
Fabricate starch block electrophoresis column.
Design and fabricate automatically times stimulator for small animals.
Fabricate adjustable plastic dissecting tray.
Fabricate plastic sedimentation blocks.

Glass Blowing:
Engrave glass tubes.
Fabricate index tubes.
Fabricate and grind glass cylinders for pumping apparatus.

Dr. Charles Maxwell:
Modify helix pitch of fraction counter and fabricate spiral plastic rack to accomodate special glass vials to be used with counter.
Fabricate miscellaneous parts for 500 curie cobalt source.
Fabricate plastic multiple bottle washer with stainless steel drain trays.

Glass Blowing:
* Fabricate 3 stage water still with automatic float valve to control water level and rate of flow. For use in radiation experiments.
* Fabricate vacuum apparatus.
Fabricate chromatography columns.
Repair irradiation flasks.

Project Description: (Continued)

Mr. Robert Swain:
Modify isodose scanner drive and fabricate portable stand for same.
Fabricate parts for dose distribution device for 3 MEV Van de Graaff.
Fabricate lead shield for scintillation detector.
Fabricate calibrated lead filters for X-ray dosage determinations.
Fabricate implant projector for demonstration purposes.
Machine work associated with maintenance of 2 MEV and 3 MEV Van de Graaffs.
Fabricate ion chamber as per instructions.

Glass Blowing:
Fabricate glass traps for 3 MEV Van de Graaff generator.

Mrs. Dorothy Peterson:
Modify and fabricate various types of pivoted mouse cages to determine X-ray response.
Modifications to 200 fiberglass animal cages.

Miss Marion Matthews:
Design and fabricate special shaking apparatus for beakers.

Dr. Peter Riesz:
Glass Blowing:
* Fabricate glass degassing apparatus for use in radiation experiments.
Fabricate glass irradiation cells.
Modify glass syringes.

Dr. James Rose:
Glass Blowing:
Fabricate glass cups with rods.
* Fabricate conical shape homogenizer tubes with conical shape plungers ground to fit.
Modify glass flasks.

Dr. Gordon Sharp:
Glass Blowing:
Fabricate pipettes.

Department of Physiology

Dr. Marco Rabinovitz:
Fabricate plastic plate holders for radioactive plate assemblies.

Project Description: (Continued)

Fabricate plating assemblies to hold glass tube and aluminum discs under pressure.

Glass Blowing:
* Develop and fabricate glass columns with one surface removable and ground to fit. For chromatography experiments.
* Develop and fabricate glass cylinders with inside surface ground to 45° angle. For evaporation experiments.

Dr. James Reid:
Modify electro-mechanical register to provide switching action at pre-determined count.
Modify Carey Recording Spectrophotometer to include 10 turn potentiometer in pen positioning mechanism.

Glass Blowing:
* Fabricate glass parts for lyophilizer.
Fabricate glass paper holder for chromatography.
* Fabricate flow-type absorption cell from quartz.

Dr. Mortimer Elkind:
Fabricate adapter rings for radiation charger.
Fabricate adapter cap for vibrating reed electrometer.
Fabricate electrode for strapilation counter.
Fabricate plastic gassing apparatus.
Fabricate microscope stage assemblies to accomodate various size petri dishes.
Design and fabricate all plastic shaking apparatus for radiation of mammalian cells in suspension.

Glass Blowing:
* Modify and rebuild 2 stage water still. Install automatic valve.
* Fabricate self-filling dispensers.
Fabricate glass cylinders.

Dr. Seymour Wollman:
Fabricate chromatography plate assembly as per specifications.

Dr. Bernard Burr:
Fabricate experimental stainless steel capillary inlet lines for model 21-201 mass spectrometer.

Glass Blowing:
* Fabricate gas sampling tubes for use with mass spectrometer.
Fabricate traps.

Project Description: (Continued)

Mr. Nathan Coffey:
Build motor driven disc assembly as per specifications.

Dr. Arnold Pratt:
Fabricate triple wall metabolism cage as per specifications.

Glass Blowing:
Fabricate gas reaction vessel with mercury pump.

Dr. Florence Millar:
Glass Blowing:
Fabricate lyophilizing bottles.
* Fabricate glass manifold with individual collecting traps.

Dr. Julius White:
Glass Blowing:
* Fabricate glass lyophilizer.
* Fabricate traps for lyophilizer.
Fabricate adaptors.

Dr. Joseph Shack:
Glass Blowing:
Fabricate pipette racks.

Dr. Herbert Kahler:
Glass Blowing:
* Fabricate glass electrodes for pH experiments.
Fabricate pipettes.

Part B included Yes /__/ No /X/

Serial No. NCI-642

1. Radiation Branch
2. Rad. Biol. Sec.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Effects of Radiation and Other Factors on Immune Response in Animals

Principal Investigator:

Falconer Smith, Ph.D.

Other Investigators:

John Owens and Gerald Ellison

Cooperating Units:

Laboratory of Physical Biology, NIAMD

Man Years:
Professional: 1/4
Other: 1-1/2
Total: 1-3/4

Project Description:

Objectives: To determine the effect of radiation on the capacity of laboratory animal to form antibody and to compare effects of other agents on the immune process.

Methods Employed: Immune responses of laboratory animals were tested before or after either total body or partial body exposure to ionizing radiation. Methods of stimulating antibody formation include altitude acclimitization followed by immunization and testing. Direct irradiation of antiserum is being carried on at doses of from 1 to 6 megarads as a part of a study of the characterization of the hemolysin antibody molecule.

Major Findings: (1) Altitude acclimitization of rats significantly increases their capacity to form antibody following immunization with sheep erythrocytes. The observed increased antibody titer does not appear to be attributable to circumstances associated

Project Description: (Continued)

with altitude exposure only. (2) Results of chronic exposures of mice to ionizing radiation at dose rates between 30 and 150r per day suggest that the capacity to form antibody after exposure is dependent on the total exposure dose and independent of dose rate at total doses of 800r or less. Groups of mice exposed to 1600r total at the rate of about 120r per 24 hours had a lower average hemolysin titer (1:15) than other mice exposed to the same total dose at the rate of about 30r/day. (3) Results of direct irradiation of antiserum show that degredation of the antibody molecule is a radiation-dose dependent phenomenon occurring at the rate of about 610 hemolytic units per 1×10^6 rads.

Significance to Cancer Research: Data which attempts to relate immunologic function with cellular injury may be expected to yield significant information in current problems associated with radiation therapy of neoplastic tissue.

Proposed Course of Project: Studies on the immunochemistry of serums of altitude acclimitized animals will include serum electrophoretic analyses, quantitative antibody determination and pathological studies of reticuloendothelial structures.

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Smith, F., Altland, P.D., and Highman, B., Effect of High Altitude Acclimitization on Formation of Sheep Erythrocyte Hemolysin in Rats, Journal of Infectious Diseases (in press).

Honors and Awards relating to this project:

None

Serial No. NCI-659*
1. Radiation Branch
2. Rad. Biol. Sec.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Physiological Processes Involved in Spontaneous and Induced Recovery from Radiation Damage

Principal Investigator:

Willie W. Smith, Ph.D.

Other Investigators:

Ilo M. Alderman

Cooperating Units:

Department of Biostatistics, School of Hygiene and Public Health, The Johns Hopkins University

Man Years:
Professional: 1
Other: 2-1/3
Total: 3-1/3

Project Description:

Objectives: To determine: (a) what factors and influences enable an irradiated animal to survive; (b) what processes determine the time of spontaneous hematopoietic recovery; (c) what treatments can alter recovery time; (d) what is the physiological basis for the induced changes in recovery time; and, (e) the characteristics of granulocyte mobilization and distribution following irradiation.

Methods Employed: Hematological and other studies are made during recovery of sublethally and midlethally irradiated animals of various ages and species, with the view of associating the observed variables with ability to survive and with the time of recovery of the various components of the hematological systems.

* Old projects are now accounted for under a single number.

Project Description: (Continued)

Various substances and procedures are tested for ability to alter survival and recovery times. When this is accomplished attempts are made to relate such effects to known or hypothetic physiological phenomena. Studies include: ability to mobilize granulocytes; resistance to experimental infection or toxin; recovery of granulocytes, lymphocytes, erythrocytes and platelets; and recovery of ability to form antibodies. Treatments used include: bacterial endotoxins and related materials; colchicine and colchicine derivatives; and other substances known to be taken up by cells of the reticuloendothelial system.

Major Findings: (a) The time of spontaneous granulocyte recovery varies with age in mice and is associated with ability to survive irradiation (reported at the fall meeting of the American Physiological Society). The relationship between age and ability to survive irradiation is different in hamsters. (b) Recovery of ability to form antibodies against homologous ascites tumor cells does not parallel recovery of lymphocytes or any other of the circulating cells studied, though it is greatly hastened by injection of spleen (but not bone marrow) cells. (c) Detailed studies of granulocyte mobilization reveal that the ratio of mobilized to nonmobilized cells is essentially the same in irradiated as in nonirradiated mice, over a range of 100 to 5,000 nonmobilized granulocytes per cu. mm (reported at the spring meeting of the Radiation Research Society). The four-fold increase following an endotoxin injection makes it very easy to demonstrate the relationship between granulocytes count and survivorship. The complexities of granulocyte distribution in the body are currently under investigation.

Significance to Cancer Research: With the increasing interest in whole-body irradiation as a therapeutic measure and in tissue and organ transplantation, the significance of studies which advance our knowledge of recovery processes and how to influence them becomes obvious. More basic significance concerns the physiology of retardation and stimulation of cell devision.

Proposed Course of Project: Attempts to understand better the physiological processes involved in hematopoietic recovery and granulocyte kinetics will be intensified. We shall try to rule in or out the possible role of mobilization in granulocyte recovery and the possible connection between the effects obtained with endotoxin and colchicine and other chemically unrelated substances in this regard. We plan also to extend our studies of spontaneous and induced hematopoietic recovery to young hamsters and to study body temperature in these animals after irradiation.

Project Description: (Continued)

Additional studies will be made in mobilization and distribution of granulocytes.

Part B included Yes/X/ No/__/

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Smith, W.W. and Hollfcoft, J., Recovery from Radiation-Induced Tolerance to Homologous Tumor, Radiation Research 13, 547, 1960.

Smith, W.W. and Cornfield, J., Utilizing Survival Time in Probit Analysis, Radiation Research 13, 540, 1960.

Honors and Awards relating to this project:

None

Serial No. NCI-674
1. Radiation Branch
2. Rad. Biol. Sec.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Synthesis of Antibody in C_3H Mice Bearing the Transplantable Plasma Cell Tumor, X-5563

Principal Investigator:

Falconer Smith, Ph.D.

Other Investigators:

John Owens and Gerald Ellison

Cooperating Units:

None

Man Years:
Professional: 1/2
Other: 1
Total: 1-1/2

Project Description:

Objectives: Characterization of the immune mechanism in mice bearing the transplanted plasma cell tumor, and to determine the combined effects of radiation and specific antibody on tumor growth and development.

Methods Employed: Standardized procedures were used to test the capacity of tumorous mice to form specific antibody both before and after irradiation of the tumor. Studies on the half life of passively transferred homologous and heterologous antibody have been carried out.

Major Findings: (1) Return of the immune response to near normal limits has been observed in tumorous mice immunized as early as 24 hours after tumor irradiation. (2) Accumulating evidence suggests that heterologous antiserum disappears from the circulation of tumorous mice more rapidly than it does from non-tumorous

Project Description: (Continued)

controls. Taken together these findings indicate that the metabolic disturbance in the degredation and synthesis of important serum components associated with the plasma-cell tumor may be altered by direct irradiation of the tumor.

Significance to Cancer Research: Analysis of the mechanism of the disturbance in globulin synthesis may lead to useful methods in the treatment of myeloma neoplasma.

Proposed Course of the Project: Isolation of the abnormal serum globulin by electrophosesis and a study of the effects of antiserum prepared against this globulin. Studies of homologous skin grafts and anaphylaxis in tumorous mice will be carried on in a further effort to clarify the mechanisms of protein synthesis by the neoplasm.

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Smith, F., Grenan, M.M. and Owens, J., Effect of a Transplanted Plasma-Cell Tumor on Antibody Formation, Journal National Cancer Institute, 25, 803-812, 1960.

Honors and Awards relating to this project:

None

Serial No. NCI-695

1. Radiation Branch
2. Rad. Biol. Sec.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Immunologic Studies on a Functional Thyroid Tumor of Rats and Immunochemical Investigations of Thyroglobulin

Principal Investigator:

Gordon Sharp, M.D.

Other Investigators:

Falconer Smith, Ph.D., Henry Metzger, M.D., John Owens, and Gerald Ellison

Cooperating Units:

Laboratory of Clinical Endocrinology, NIAMD

Man Years:
Professional: 1-1/2
Others: 1
Total: 2-1/2

Project Description:

Objectives: (1) Using immunochemical techniques, studies of the effects of thyroglobulin of rat origin on the growth, development and pathology of a functional thyroid tumor are being carried on. (2) Tests of the antegenicity of degredation products of thyroglobulin.

Methods Employed: Species specific thyroglobulin was prepared from donor rat thyroid glands and from functional tumors. These preparations have been used as antigens for administration to host rats in which thyroid tumors were transplanted simultaneously, before or after immunization. Tumor growth rates and incidence were observed. Immunochemical tests of the thyroglobulin and its degredation products were made as well as quantitative estimates of circulating antithyroglobulin antibody in the host animals.

Project Description: (Continued)

Major Findings: (1) Preliminary estimates of serum antibody following administration of thyroglobulin with Freund's adjuvant show that antibody reaches peak titer about 3 weeks after immunization. (2) Immunization of rats 3 weeks prior to tumor transplant resulted in a significant delay in the rate of tumor growth and may be associated with a reduction in the numbers of tumors produced. (3) Circulating antithyroglobulin does not appear to be associated with abnormal tumor pathology.

Significance to Cancer Research: Specific biochemical products of neoplastic cells which can be used as antigens may prove to be important chemotherapeutic adjuncts in radiation therapy.

Proposed Course of Project: Repetition of the preliminary results will be followed by the use of passively transferred antithyroglobulin in combination with tumor irradiation as a treatment of transplanted tumors.

Part B included Yes [ ] No [X]

Serial No. NCI-624
1. Radiation Branch
2. Radiation Physics Sect.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Radiation Injury and Repair

Principal Investigator:

H. L. Andrews

Other Investigators:

Major James Shively

Cooperating Units:

Walter Reed Army Medical Center

Man Years:

Total: 1/3
Professional: 0
Other: 1/3

Project Description:

Objectives: To gain insight into mechanisms by which ionizing radiations affect living tissues.

Methods Employed: Repair processes at high x-ray doses were studied by dose fractionation. A second phase involves studies on swine, animals of interest because their size approximates that of man.

Major Findings: Dose-response data for swine have been obtained from the mid-lethal range up to doses of several thousand roentgens. Offspring from heavily irradiated parents appear to have a dose-response curve identical with that of pigs from non-irradiated parents. This project has been terminated and a manuscript submitted for publication.

Part B included Yes ☐ No ☒

Serial No. NCI-628
1. Radiation Branch
2. Radiation Physics Sect.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Dosimetry of High Energy Radiation

Principal Investigator:

H. L. Andrews

Other Investigators:

L. Cameron

Cooperating Units:

None

Man Years:

None

Project Description:

Objectives: To develop methods and instruments for measuring high intensity pulsed x-ray beam.

Methods Employed: No progress was made on this project during 1960 and it can probably be considered to be terminated.

Part B included Yes ☐ No ☒

Serial No. NCI-629
1. Radiation Branch
2. Rad. Physics Sect.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

The Effect of Ionizing Radiation on Amino Acids

Principal Investigator:

Charles R. Maxwell, Ph.D.

Other Investigators:

None

Cooperating Units:

Energy Metabolism Section, Laboratory of Physiology, NCI

Man Years: (calendar year 1960)
Professional: 1
Other: 1/2
Total: 1-1/2

Project Description:

Objectives: General: The objective of this project is to determine the mechanism of the chemical reactions induced by ionizing radiation in aqueous solutions of amino acids. This is part of a long range program to accumulate information on simple systems of biological interest so that general principles may be ascertained and applied to complex systems which are not amenable to thorough, direct investigation.

Specific: 1) To search for, identify and determine the yields of some unknown compound or compounds produced in the irradiation of aqueous glycine. Evidence for the existence of these compounds is the lack of a material balance for the overall reaction, and the presence of some unidentified peaks observed on an ion-exchange chromatograph when C-14 labeled glycine is irradiated in the absence of oxygen. 2) To study in more detail the mechanism for the formation of acetic acid. 3) To install a Co-60 radiation facility.

Project Description: (Continued)

Methods Employed: Solutions of amino acids are irradiated with ionizing radiation and then analyzed for the produds formed. Since the products are sensitive to radiation it is necessary to determine the yield of each product as a function of dose to as low as dose as possible and to extrapolate these values to obtain the initial yield of the product at very low doses before secondary reactions distort the picture. For this work isotopically labeled compounds are used a great deal. Associated techniques and methods include paper chromatography, ion exchange and partition chromatography, gas chromatography, paper strip and liquid scintillation counting as well as the use of the mass spectrograph in the Laboratory of Physiology.

Once the products of the simple water-amino acid-radiation system are found the effect of the introduction of other chemicals into the system are studied. Substances are selected, in accordance with the rapidly expanding concepts of radiation chemistry, which are thought to accentuate or repress one or more of the various intermediate processes by which radiation is believed to produce its effects.

Since the final chemical (and ultimate biological) effect is the result of the formation of a complex microscopically-heterogenous mixture of excited molecules, H_2O_2, H and OH free radicals in discrete "tracks", the influence of the physical distribution of these active intermediates as well as possible differences in relative abundance and/or chemical nature is investigated by using different sources of radiation. To date 50 KV X-rays, 150 KV electrons, and 5 Mev alpha particles have been used.

One of the more important, if not the most important, active intermediates is the OH free radical. The role of this radical in the absence of the H free radical and the host of excited molecules in the ionization track is studied by chemically producing this radical in the system being studied. To date this has been accomplished by the action of Fe^{2+} and H_2O_2.

Major Findings: Glycolic acid, $CH_2OHCOOH$, has been added to the previous list of twelve compounds known to be formed when solutions of glycine are irradiated with X-rays. Its initial specific yield has been studied as a function of concentration in both the absence and presence of dissolved oxygen. This data is qualitatively similar to such data for HCHO and suggests a similar mechanism for formation. In an oxygen-free system 0.15 molecules of $CH_2OHCOOH$ are formed for each 100 electron volts of energy absorbed in one molar glycine solution. In the presence of oxygen this value is increased to 0.3 (Approximately 4.5 molecules of glycine are destroyed for each 100 ev absorbed in each case). These yields,

Project Description: (Continued)

though small, are of specific interest because glycolic acid is reported to be the principle product from the ultraviolet photolysis of aqueous glycine.

The mechanism for the formation of acetic acid seems to be fairly well established.

According to the mechanism proposed by Weeks and Garrison

Boyd M. Weeks and Warren M. Garrison, Rad. Research 9: 291, (1958).

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acetic acid is formed by the reactions:

$$1)\quad H + NH_2CH_2COOH \rightarrow NH_3 + \cdot CH_2COOH$$

$$2)\quad \cdot CH_2COOH + NH_2CH_2COOH \longrightarrow CH_3COOH + NH_2\dot{C}HCOOH$$

In D_2O the corresponding reactions would be:

$$1)\quad D + ND_2CH_2COOD \rightarrow ND_3 + \cdot CH_2COOH$$

$$2)\quad \cdot CH_2COOD + ND_2CH_2COOD \longrightarrow CH_3COOD + ND_2\dot{C}HCOOD$$

and the acetic acid formed (after the removal of the exchangeable D on the carboxy group) should be CH_3COOH.

Last year mass spectrographic analysis of the acid isolated showed a mixture of CH_2DCOOH and CH_3COOH, indicating a second method for the formation acetic acid. This year studies of the yields of these two products as a function of dose has shown the yield of CH_2DCOOH to increase with dose with a corresponding decrease in the CH_3COOH yield. It would thus appear that the source of CH_2DCOOH is a secondary reaction such as

$$3)\quad \cdot CH_2COOH + \text{labeled product} \rightarrow CH_2DCOOH$$

competing with 2) as the concentration of this product increases with increasing dose. At low doses where only glycine is present acetic acid is formed by 1) and 2).

A major effort this year has been the procurement, supervision of fabrication, and installation of a cobalt-60 radiation facility. Installation was completed in September and we now have an easily

Project Description: (Continued)

operated, versatile 600 curie Co-60 facility with a temperature controlled radiation chamber 16" in diameter by 14" high. With proper placement of samples dose rates from 300 to 5000 roentgens per min. may be used.

Significance to Cancer Research: Although not directly connected with Cancer research, it is of great interest because it seeks an understanding of the mechanism for the effects of radiation which is used empirically as a tool in theclinical treatment and laboratory study of cancer.

Proposed Course of Project: Work will continue in an effort to fully elucidate the mechanism of the effect of radiation on glycine.

Specifically the work will be directed toward:

a) Identifying and determining the unknown product indicated by an incomplete material balance.

b) An investigation of the O_2 present system as well as the O_2 free system upon which our efforts in the past have been primarily concentrated.

c) An investigation of the effect of pH upon both the O_2 free and O_2 present system.

Part B included Yes/ ___ / No /X/

Serial No. NCI-637
1. Radiation Branch
2. Radiation Physics Sect.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Development of High-Intensity X-Ray Source

Principal Investigator:

H. L. Andrews

Other Investigators:

Robert W. Swain

Cooperating Units:

None

Man Years:

None

Project Description:

Objectives: To obtain high intensity beams of x-rays, pulsed or steady from the 3 Mev generator.

Methods Employed: No progress was made on this project during 1960 and it can probably be considered to be terminated.

Part B included Yes ☐ No ☒

Serial No. NCI-680
1. Radiation Branch
2. Radiation Physics Sect.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Radioactive Isotope Administration by Iontophoreses

Principal Investigator:

H. L. Andrews

Other Investigators:

Louis Cameron and Dorothy C. Peterson

Cooperating Units:

None

Man Years:

Total: 1
Professional: 2/3
Other: 1/3

Project Description:

Objectives and Methods Employed: A variety of isotopes were administered through the skin of guinea pigs by the application of electric current. Isotope distributions were determined by assaying skin sections and pertinent organs. The distribution of P^{32} seemed of sufficient interest to warrant an attempt at tumor treatment. Walker sarcomas in rats were treated by the local application of P^{32} by electric current.

Major Findings: No work was done on this project during 1960 and it can be considered to be terminated.

Part B included Yes [ ] No [x]

Serial No. NCI-697
1. Radiation Branch
2. Rad. Physics Sect.
3. Bethesda, Md.

PHS-NCI
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

The Abstraction Reactions of Hydrogen Atoms in the Radiolysis of Aqueous Organic Solutions. Part II. The Effect of pH on the Radiolysis of Aqueous Methyl-d_3 Alcohol. Evidence for the Existence of Two Kinds of Hydrogen Atoms.

Principal Investigator:

Peter Riesz, Ph. D.

Other Investigators:

Bernard E. Burr, Charles R. Maxwell, Ph. D., and Alton R. Rollins

Cooperating Units:

Energy Metabolism Section, Laboratory of Physiology,
National Cancer Institute

Man Years (calendar year 1960)
Professional: 1
Other: 1/6
Total: 1-1/6

Project Description:

Objectives: The action of ionizing radiation on aqueous solutions has been explained in recent years on the hypothesis that hydrogen atoms, hydroxyl radicals, hydrogen gas and hydrogen peroxide are the only primary products. Recent work by A.O. Allen and J. Weiss has indicated that at least two different kinds of stoichiometrically equivalent "H atoms" play a role in the radiation chemistry of water due to the equilibria.

$$\bar{e} \overset{H^+}{\rightleftharpoons} H \overset{H^+}{\rightleftharpoons} H_2^+$$

It seems likely that the so-called H atom produced by the irradiation of water may be the solvated electron which can react with the hydronium ion to give the true H atom. Any of these "H atoms" may react with saturated organic solutes by abstracting H atoms from carbon, nitrogen, sulfur and oxygen to produce hydrogen and an organic free radical.

Project Description: (Continued)

For solutes which have more than one site at which H atom abstraction may occur, there is at present insufficient information for predicting the site or sites of reaction. The objectives of this project are to study the mechanism of abstraction reactions for such solutes and thus to determine the nature of the organic free radicals produced. In addition we have attempted to distinguish between the chemical effects of hydrogen atoms and solvated electrons.

Methods Employed: When aqueous methyl-d_3 alcohol is exposed to Cobalt gamma radiation in the absence of oxygen, the following reactions occur:

1. $H + CD_3OH \xrightarrow{kc} HD + \dot{C}D_2OH$

2. $H + CD_3OH \xrightarrow{ko} H_2 + \dot{C}D_3O$

In addition there is a primary molecular hydrogen yield (g_{H_2}) which arises from the recombination of "H atoms" in a series of small regions of high radical concentration (known as "spurs"). The "H atoms" which escape recombination and diffuse out of the spurs react with the organic solute. From the observed yields of H_2 and HD [$G(H_2)$ and $G(HD)$], the ratio of the rate constants, ko/kc, can be calculated since

$$\frac{ko}{kc} = \frac{G(H_2) - g_{H_2}}{G(HD)}$$

Techniques for measuring H_2 and HD in the concentration range 10^{-7} to 10^{-6} moles/liter were developed. The amount of CD_2HOH present in CD_3OH was determined by nuclear magnetic resonance techniques and was found to be 2.0%.

Since aqueous methanol neither loses nor gains a proton in the pH range 0.4-10, the ratio ko/kc will be constant over this pH range provided only one kind of H atom is involved in abstraction reactions, but may be expected to change with pH if more than one kind of "H atom" reacts with methanol.

Major Findings: Previous work on the abstraction reactions of D atoms produced by irradiation of D_2O with 230 kvp X-rays was extended. The new solutes investigated were methanol-d, ethanol-d, isopropanol-d, serine-d, formic acid-d and acetic acid-d. The primary molecular hydrogen yield for 230 kvp X-rays was determined by irradiation of deaerated bromide solutions and was found to be $g_{D_2} = .60 \pm .02$ for H_2O, $g_{D_2} = .49 \pm .02$ for D_2O. For cobalt γ

Project Description: (Continued)

radiation we find g_{D_2} = .45 ± .02 in agreement with the accepted literature value.

For all new solutes the observed $G(D_2)$ values were equal or smaller than the observed primary molecular yield of 0.49 ± .02. This shows that D atoms abstract very predominantly from carbon and not from oxygen. However, while $G(D_2)$ for 0.1 M glycine, acetamide, glycylglycine and formic acid is equal to 0.40 ± .02, the values for methanol, ethanol, isopropanol and serine were .50, .47, .45 respectively. Since the lowering of the D_2 yield below the primary molecular yield is presumably due to reactions of the 0.1 M solutes with D atoms (or their percursors) inside the "spurs", the absence of this effect for methanol suggested that some abstraction from oxygen was taking place. The latter process would be expected to be more important for CD_3OH than CH_3OD since O-D and C-D bonds are stronger than O-H and C-H bonds.

H_2 and HD yields from Cobalt gamma irradiated solutions of 0.1 M methyl-d_3 alcohol in light water were measured over the pH range 0.4-6. The ratio of the rate of abstraction from oxygen to the rate of abstraction from carbon, ko/kc, was found to be constant and equal to 0.25 ± .01 in the pH range 0.40-5.0. However at pH 6 ± .2 the ratio ko/kc is equal to 0.58 ± .02. Since the solute methanol neither loses nor gains a proton in this pH range at least two kinds of hydrogen atoms, differing in chemical reactivity, must take part in these abstraction reactions. These results may be interpreted in terms of a competition of methanol and hydronium ions for solvated electrons.

Significance to Cancer Research: This project contributes to an understanding of the primary processes in the irradiation of water and of the free radical reactions which play an important role in the irradiation of biological systems.

Proposed Course of Project: The effects of methanol concentration, pH and dose on the H_2 and HD yields are being further investigated. It is hoped that other systems which differentiate chemically between the different kinds of hydrogen atoms may be found.

Part B included Yes [ ] No [X]

Serial No.NCI-952
1. Radiation Branch
2. Radiation Physics Sect.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Sensing and Response to Radiation

Principal Investigator:

H. L. Andrews

Other Investigators:

D. C. Peterson

Cooperating Units:

None

Man Years:

Total: 1 2/3
Professional: 1 1/3
Other: 1/3

Project Description:

Objectives: To study the ability of animals to sense radiation, determine their reaction to it, and study the mechanisms eliciting the response.

Methods Employed: Mice are free to move in a container one half of which is exposed to an x-ray beam. The animals choice patterns are being studied as functions of dose rate and other variables.

Major Findings: Mice can sense an x-ray beam under some circumstances. The subsequent behavior depends upon dose rate. It appears that ozone is implicated in the behavior and perhaps in some other reactions to radiation.

Part B included Yes ☐ No ☒

Serial No. NCI-953
1. Radiation Branch
2. Rad. Biol. Sec.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Quantitative Cell Survival - Radiation Dose Relationships in a Mammalian Tumor System

Principal Investigator:

Roger J. Berry, M.D.

Other Investigators:

None

Cooperating Units:

Department of Radiology,
U.S. Naval Medical Center
Bethesda, Md.

Biology Division
Brookhaven National Laboratories
Upton, LI, N.Y.

Man Years (calendar year 1960)
Professional: 1/2
Other: 1
Total: 1-1/2

Project Description:

Objectives: A quantitative system for the determination of cell survival -- radiation dose relationships has been developed using the ascites form of a transplantable lymphocytic leukemia in the DBA/2 strain mouse. This system provides a method for studying changes in radiosensitivity related to alteration in oxygen tension, ionization density of the radiation (LET), or as induced by pharmacological agents.

Methods Employed: Serial dilutions of the ascitic fluid of a tumor-bearing donor mouse are injected into groups of recipient mice to determine the 'TD-50', i.e. the number of tumor cells needed

Project Description: (Continued)

to produce 'takes' in 50% of recipients. The system offers freedom from host-immunity factors, as control tumor suspensions show repeatably low TD-50 values (2-3 cells).

Major Findings: A dose-response curve for 3-MEV x-rays has been established; it is of the '2-hit exponential' type. If hydrogen peroxide, to final concentration 0.06 M, was given intraperitoneally to donor tumor-bearing mice immediately prior to irradiation, a steeper dose-response slope was obtained. The 'oxygen enhancement ratio' approximated 2.5:1. Preliminary experiments have shown that fractionation of total radiation dose increases the number of cells surviving. The administration of 5-bromodeoxyuridine or 5-iododeoxyuridine to tumor-bearing donor animals (at doses of 100-300 mgm/Kg/day) for 4 days prior to irradiation produced a marked decrease in the number of surviving tumor cells in preliminary experiments. A dose-response curve for fission-spectrum fast neutrons has been established.

Significance to Cancer Research: This method permits study of the parameters of radiation effect upon the reproductive integrity of tumor cells in the intact mammalian host, rather than in tissue-culture explants or lower-order biological systems. It provides a relatively rapid method for evaluation of radiation potentiation or protection by pharmacological agents.

Proposed Course of Project: The potentiation of radiation effect by incorporation of halogenated pyrimidine deoxyribosides into the tumors of donor animals requires confirmation. The effect of dose-fractionation on the radiation-plus-drug system will be studied to determine whether the 'hit number' (extrapolation number) is altered by building these abnormal 'blocks' into the tumor DNA. The radiosensitivity of a tetraploid analogue of the tumor presently being studied will be determined, as will the time-dose relationship of administration of DNA precursor analogues. Studies of effects of high-LET radiation (e.g. fast neutrons, accelerated charged particles) will be extended.

Part B included Yes [ ] No [X]

Serial No. NCI-954
1. Radiation Branch
2. Radiobiology Sec.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Serum Lactic Acid Dehydrogenase Activity in Experimental Animals Bearing Transplantable Tumors.

Principal Investigator:

Roger J. Berry, M.D.

Other Investigators:

Robert Greenfield, M.D.

Cooperating Units:

Laboratory of Biochemistry, NCI

Man Years

Professional: 1/4
Other: none
Total: 1/4

Project Description:

Objectives: A serum-transmissible factor capable of elevating the serum lactic acid dehydrogenase (LAD) activity in recipient animals has recently been reported in the sera of tumor-bearing mice. The present studies were designed to extend these observations to other species of experimental animals bearing transplanted tumors, and to use ionizing radiation as a tool for investigating the mechanism of transmission of this enzymic abnormality.

Methods Employed: Determination of serum lactic acid dehydrogenase has been done by a DPNH-disappearance spectrophotometric method developed by Greenfield.

Major Findings: We have confirmed the earlier finding that serum LAD activity is increased in mice bearing transplanted tumors, and

Project Description: (Continued)

have extended the original tumor list studied to include 3 additional mouse strains and 6 additional mouse tumors. Mice which had received cell-free tumor extracts and showed no signs of gross tumor 2-3 months later still showed increased serum LAD activity. In preliminary experiments, a slight elevation in serum LAD activity was noted in guinea pigs bearing a transplanted lymphocytic leukemia. Rats bearing several tumors of widely differning morphologic types (including one tumor of known viral etiology which had produced LAD elevation in mice) did not evince increased serum LAD activity even in presence of gross tumor. In a single thyroid tumor of rats, serum LAD was depressed in tumor-bearing animals and returned to normal in animals in which established tumors had been suppressed by administration of an heterologous antibody to the thyroglobulin produced by the tumor.

Significance to Cancer Research: An enzymic defect has been demonstrated in a large number of transplantable mouse tumors and in the sera of animals bearing any of these tumors. This defect is detectable prior to the development of gross tumor and can be transmitted by cell-free extracts which are incapable of producing tumor in recipient animals. The mechanism seems to be species-specific; its relationship to carcinogenesis remains to be evaluated.

Proposed Course of Project: Experiments are in progress to determine whether the enzymic defect resulting in increased LAD activity can be transmitted by tumor suspensions which have been irradiated to doses adequate to destroy virus activity but insufficient to cause major protein denaturation. Studies of the species-dependence and apparent inversion of this mechanism in the rat will be extended.

Part B included Yes/ / No/X/

Serial No. NCI-2-650(c)
1. Radiation Branch
2. Rad. Ther. Serv.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Service Radiation (Clinical Therapy)

Principal Investigator:

Kirkland C. Brace, M.D.

Other Investigators:

J. Robert Andrews, M.D.

Cooperating Units:

None

Man Years:
Professional: 2/3
Other : 1-1/2
Total: 2-1/3

Project Description:

Objectives: To provide radiation therapy for those patients requiring such treatment in the course of the disease for which they are being investigated by NIH research groups other than the Radiation Branch.

Patient Material: A total of 200 consultations were seen in the various departments of this Institution during the past 12 months. A total of 176 courses of radiation therapy were administered.

Methods Employed: A wide range of photon energies were used and various methods of application were employed. Following are the total number of courses of treatments administered in this department at the various energies noted:

192 courses of 2 Mev irradiation
2 courses of 250 kilovolt radiation
9 courses of 140 kilovolt radiation
2 courses of 60 kilovolt radiation

Project Description: (Continued)

7 courses of electron beam therapy
5 radium applications both intracavitary and interstitial

Major Findings: Response to radiation therapy has been consistent with responses reported by others.

Proposed Course of Project: To continue.

Part B included Yes / / No /X/

Serial No. NCI-652 (C)
1. Radiation Branch
2. Rad. Ther. Serv.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Childhood Cancer

Principal Investigator:

Kirkland C. Brace, M.D.

Other Investigators:

Louis V. Avioli, M.D., Haskins Kashima, M.D., and Michael Lazor, M.D.

Cooperating Units:

None

Man Years:
Professional: 1
Other: 0
Total: 1

Patient Days: 600 approx.

Project Description:

Objectives: The objective of this program is to collect data on the malignant disease of children.

Patient Material: All children under the age of 18 who have malignant tumors.

Methods Employed: Standard radiation therapy techniques and steroids.

Major Findings: During the past year an effort has been made to collect cases of Hand-Schuller-Christian disease. This disease has been a radiation therapeutic problem in the past with relatively poor results. We have undertaken to treat 12 advanced cases with high doses of steroids. There has been moderate success as measured by regression of soft tissue manifestation. Only a prolonged follow-up will determine how successful this type of treatment is.

Project Description: (Continued)

Proposed Course of Project: We will not take any new cases of Hand-Schuller-Christian's disease on this study but will continue to follow the material presently available. Efforts will continue to collect additional cases of childhood cancer of other types.

Part B included Yes [ ] No [X]

Serial No. NCI-653(C)
1. Radiation Branch
2. Rad. Ther. Serv.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Radiation Effect Study

Principal Investigators:

Louis V. Avioli, M.D. and Kirkland Brace, M.D.

Other Investigators:

Michael Lazor, M.D., E. Cotlove, M.D. and Haskins Kashima, M.D.

Cooperating Units:

None

Man Years:
Professional: 1/2
Other: 0
Total: 1/2

Project Description:

Objectives: Renal function is specifically evaluated before and following therapeutic abdominal irradiation.

Patient Material: Patients with abdominal neoplasia requiring therapeutic abdominal irradiation, e.g. ovarian carcinoma, retroperitoneal metastatic disease, etc.

Methods Employed: Renal function is evaluated via dehydration studies, Addis counts, serum blood urea nitrogen and creatinine determinations, intravenous pyelograms and more specifically, glomerular filtration rates, renal plasma flow and maximal tubular capacity for para-amino hippuric acid. These studies are performed before and following fractionated doses of abdominal irradiation.

Major Findings: To date there has been no consistent acute effect of single doses of radiation in the range of 400-500 rads for the parameters noted above in the four patients evaluated.

Project Description: (Continued)

Proposed Course of Project: The study will continue as outlined in order to appreciate the chronic effect of renal irradiation using the specific renal testing parameters noted above.

Part B included Yes [ ] No [X]

Serial No. NCI-2-654(c)
1. Radiation Branch
2. Rad. Ther. Serv.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Time as a Modifier of Clinical Radiation Response

Principal Investigator:

J. Robert Andrews, M.D.

Other Investigators:

Kirkland C. Brace, M.D. and Haskins Kashima, M.D.

Cooperating Units:

None

Man Years:
Total: 1/4
Professional: 1/8
Other: 1/8

Patient Days: 200

Project Description:

Objectives: To determine the effect of change in the rate of radiation administered upon the therapeutic effect of X rays.

Patient Material: Patients with inoperable carcinoma of the head and neck.

Methods Employed: This is a continuation of NCI-2-654(c). The theoretical dosimetry and general clinical aspects of the project have been previously reported. The methods are those of standard radiation therapy techniques.

Major Findings: The prolonged radiation study program of the past several years has been discontinued because of disappointing results. We have now undertaken to examine the rapid treatment aspect of radiation therapy. At the present time four patients have been treated with a single dose of radiation administered over an interval of about

Project Description: (Continued)

forty minutes. The results of this treatment have been most satisfactory for its immediate effect on the tumor and essentially without complication. The ultimate effect on survival will of course require some time to determine.

Proposed Course of Project: We plan to continue treating all head and neck cancers available on the program until we have collected at least 40 cases.

Part B included Yes /__/ No /X/

Serial No. NCI-2-656(c)
1. Radiation Branch
2. Rad. Ther. Serv.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Distribution of S-35 in Human Tissues and Tumors and the Clinical Effects of Large Doses of S-35

Principal Investigator:

J. Robert Andrews, M.D.

Other Investigators:

Kirkland C. Brace M.D., Haskins Kashima, M.D., Michael Lazor, M.D. Richard Swarm M.D., and Hazel Gump, A.B.

Cooperating Units:

Department of Anesthesiology, Surgery Branch, NCI, Pathologic Anatomy Branch, NCI, and National Naval Medical Center

Man Years (calendar year 1960): Patient Days: 150
Professional: 1/4
Other: 1/4
Total: 1/2

Project Description:

Objectives and Methods Employed: The conceptual aspects of the project remain the same as reported in detail as NCI-2-656(c) for the calendar year 1959. The study has been severely handicapped because appropriate patients have not become available.

Patient Material and Major Findings: Since the last report two additional cases of chondrosarcoma have become available for study on this program. Both of these are young men with extensive recurrent disease following surgery. Of the previous cases studied, only one survives, and she has shown no change in her disease over a period of two years.

From our studies it has been apparent that the limiting dose is determined by bone marrow depression. In an effort to overcome this problem one of these patients had a homologous marrow transfusion one week following administration of the sulfur. The

Project Description: (Continued)

marrow was stored and prepared for us by Dr. McFarland of the Navy Medical Center.

The other patient was given the S-35 following occlusion of arterial circulation and cooling of three limbs to 1-1/2° C. for six hours following the administration of the sulfur. At this time the arterial occlusion was removed and the tissue allowed to return to normal temperatures.

These patients are now in the initial phase of the experiment and no firm conclusions can be drawn at this time.

Proposed Course of Project: These studies will be continued as suitable case material becomes available.

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Andrews, J.R., Swarm, R.L., Schlachter, L., Brace, K.C., Rubin, P., Bergenstal, D.M., Gump, H., Siegel, S., Swain, R.W., The Effects of One Curie of Sulfur 35 Administered Intravenously as Sulfate to a Man with Advanced Chondrosarcoma, The American Journal of Roentgenology, Radium Therapy and Nuclear Medicine, 83, 123-134, 1960.

Honors and Awards relating to this project:

None

Serial No. NCI-2-660(C)

1. Radiation Branch
2. Rad. Ther. Serv.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Clinical Radiation Dosimetry

Principal Investigator:

Robert W. Swain

Other Investigators:

None

Cooperating Units:

None

Man Years (calendar year 1960):
Professional: 1/3
Others: 1
Total: 1-1/3

Patient Days (calendar year 1960):

Project Description:

Objectives: To develop methods for and to determine radiation dosage distributions for various types of radiation therapy used in the treatment of patients and to devise methods for improvement of radiation distribution within the patient.

Methods Employed: Chemical and ionization chambers for electron beam therapy. Ionization chambers and photographic methods for Supervoltage X-ray Therapy.

Major Findings: Work on the isodose plotter has been resumed. Preliminary experimental work indicated the need for a major change in design which has been accomplished. However, some minor changes are still required. It was found that the response time of the mechanical components was too fast for the electronic components. The response time of the mechanical has been reduced and the plotter is ready for further tests.

Preliminary work is being done in x-ray scintillation spectroscopy using the Tracerlab Gamma-ray Spectrometer. Resolution and

Project Description: (Continued)

stability of the spectrometer is being checked with the possibility of using it on the 250 KV x-ray units.

Proposed Course of Project: Work will continue on the isodose plotter until completed. A3x3 crystal will be used with the 256 -channel analyzer as a preliminary study of x-ray spectroscopy with the 2 MV generators.

Other work in dosimetry will be carried on as the needs arise.

Part B included Yes [ ] No [X]

Serial No. NCI-696(C)
1. Radiation Branch
2. Rad. Ther. Serv.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Studies on Bronchogenic Carcinoma; Comparison of Combinations of Chemotherapy and X ray with X ray Alone

Principal Investigator:

Kirkland C. Brace, M.D.

Other Investigators:

J. Robert Andrews, M.D. and Haskins Kashima, M.D.

Cooperating Units:

Chemotherapy Service, General Medicine Branch, NCI

Man Years:
Professional: 1/4
Other: 1/4
Total: 1/2

Patient Days: 60

Project Description:

Objectives: Investigation of the treatment of cancer with combination of drugs and irradiation.

Methods Employed: Treatment of patients with various drugs and standard radiation therapy techniques.

Patient Material: Patients with bronchogenic carcinoma and with bilateral pulmonary metastases and malignant melanoma.

Major Findings: During the past several years this study has been combined with the nitrogen mustard and radiation as part of the program of the Eastern Hospital Group. The preliminary results of this study have been very disappointing and we have discontinued treating cases on this protocol. There have been several reports of the combined effect of radiation and Actinomycin-D in the treatment of cancer. We have chosen to study this effect using the patient as his own control. Patients with bilateral

Project Description: (Continued)

lesions were treated with radiation on one side and radiation and Actinomycin-D on the other. The initial response observed on three cases indicated that the Actinomycin increases the skin effect of the radiation but there was no detectable difference in the tumor response.

Proposed Course of Project: There is great difficulty in finding suitable cases for this study but the project will continue as they become available.

Part B included Yes [ ] No [X]

Serial No. NCI-950(C)
1. Radiation Branch
2. Rad. Ther. Serv.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Radioactive Renogram

Principal Investigator:

Louis V. Avioli, M.D.

Other Investigators:

None

Cooperating Units:

None

Man Years:
Professional: 1
Other: 0
Total: 1

Project Description:

Objectives: An attempt is being made to evaluate the effectiveness of the radioactive renogram in diagnosing renal disease.

Patient Material: Includes patients with carcinoma of cervix or bladder, unilateral renal disease with hypertension and chronic renal disease of unknown etiology. Studies are also being made of patients who received renal radiation as a consequence of therapeutic abdominal radiation for carcinoma of ovary, peritoneum, etc.

Methods Employed: Simultaneous bilateral excretion of a radioactive (I^{131}) renal specific dye (hippuric acid, miokon etc.) is appreciated over each kidney following the intravenous injection of the radioactive substance. The results are compared with standard renal evaluating programs, (e.g. IVP, creatinine clearance, renal concentration tests, PSP excretion, arteriograms).

Projection Description: (Continued)

Major Findings: The radioactive renogram technique appears to be a sensitive indicator of renal function. Renal vascular stricture, hydronephrosis and obstruction, and tubular disease have been well appreciated using this technique.

Proposed Course of Project: It is intended that the study will continue with similar aims and purposes in an attempt to further evaluate the effectiveness of this approach in evaluating renal disease.

Part B included Yes/ / No/X/

Serial No. NCI-951(C)
1. Radiation Branch
2. Rad. Ther. Serv.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Metabolic Studies

Principal Investigator:

Louis V. Avioli, M.D.

Other Investigators:

Michael Lazor, M.D.

Cooperating Units:

General Medicine Branch, NCI

Man Years:
Professional: 1
Other: 0
Total: 1

Project Description:

Objectives: To investigate calcium and phosphorous metabolism in patients with osteolytic or osteoblastic metastatic disease before and after definitive therapeutic procedures.

Patient Material: Patients with metastatic bone disease, e.g. multiple myeloma, breast carcinoma, prostatic carcinoma.

Methods Employed: Use of radioactive calcium tracer techniques and complete metabolic balance regimens.

Major Findings: Bone formation and bone resorption are markedly elevated in patients with lytic and blastic bone lesions. Effective therapeutic regimens decrease the amount of bone resorption and appear to stimulate reparative processes in bone. External counting techniques also appear to be sensitive indicators of localized bone metabolism during the course of the disease processes in lytic or blastic bone lesions.

Project Description: (Continued)

Proposed Course of Project: We propose to continue the project concentrating on magnesium tracer studies as well. We also hope to further delineate the effectiveness of these techniques in appreciating the course of metastatic bone disease.

Part B included Yes/ / No/X/

Serial No. NCI-955(C)

1. Radiation Branch
2. Rad. Ther. Serv.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title:

Whole Body Irradiation Study

Principal Investigator:

J. Robert Andrews, M.D.

Other Investigators:

Kirkland Brace, M.D., Jack Levin, M.D. and Haskins Kashima, M.D.

Cooperating Units:

General Medicine Branch, NCI

Man Years:
Professional: 1
Other: 0
Total: 1

Project Description:

Objectives: To investigate the effect of whole body irradiation on some aspects of the iron metabolism of the bone marrow and upon the peripheral blood.

Patient Material: The patients selected for this program fall into two groups. a) Generalized Hodgkin's disease and other lymphoma's. b) Chronic lymphocytic leukemia.

Methods Employed: Irradiation of the whole body with 2.5 MEV x-rays. Determination of plasma iron turnover with labeled iron-59.

Major Findings: The patient has been treated with 2.5 MEV x-rays of the 3 MEV generator which provides very uniform whole body exposure. The maximum dose variation through the thickest part of the body does not exceed 2%. The results show that depression in the peripheral count following 100r

Project Description: (Continued)

is very profound and reaches a maximum at 28 days for all the formed elements. The plasma iron disappearance has proved to be a most sensitive index of bone marrow effect. This plasma iron disappearance shows a maximum depression 2 days following treatment and returns to near normal a week after exposure. This is fdlowed by a second depression about the 4th week associated with fall in the peripheral counts.

The effect on the lymphocytic leukemia cases has been most satisfactory. The two cases treated are now asymptomatic with near normal blood counts 6 months after treatment.

Proposed Course of Project: We propose to continue the project with investigation of the effect of lesser doses on the plasma iron disappearance time. This may prove to be one of the most sensitive indexes available for early marrow damage. The results on the chronic lymphocytic leukemia suggest that more of this type of case should be included to determine how permanent this effect may be. The possibility of retreatment remains untried.

Part B included Yes [X] No [ ]

Serial No. NCI-955(C)

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Levin, J., Andrews, J.R., Berlin, N.I., The Effect of Total Body Irradiation on Some Aspects of Human Iron Metabolism, Journal Clinical Investigation.

Honors and Awards relating to this project:

None

Serial No. NCI-10-750 (c)
1. Surgery Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Removal of Cancer and Other Tissues for Histologic, Biochemical and Other Studies as Required by Scientists and Other Investigators for Correlated Studies.

Principal Investigator: Dr. Robert R. Smith

Other Investigators: Dr. Alfred S. Ketcham, Dr. John West, and Dr. Robert Madden

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1
Professional: 1
Other: 0

Patient Days (calendar year 1960):

Project Description:

Objectives:

The object of this project is to make available human tissue for histologic, histochemical, biochemical and biophysical studies to scientists working in various branches of the National Cancer Institute, who require tissue for studies. In addition, it implies furnishing general surgical coverage of patients admitted to other branches of the NCI and/or other institutes, and assisting with their study projects. As a rule, such studies are done as cooperative projects but on occasions, specific types of tissue are required for specific studies. For example, one investigator requested that specimens of skin and its associated neoplasm be made available for keratinization studies, and various muscle biopsies and/or biopsies of liver tissue under specific conditions have been requested. These materials are usually obtained during operations for standard therapeutic reasons.

Methods Employed:

Methods employed are standard surgical procedures.

Patient Material:

Material for this project is obtained mostly from patients

hospitalized for study in other projects in other branches or institutes. Occasionally, it is necessary to admit patients for specific studies in which a specific type of tissue is obtained. There have been several examples of this, mostly at the request of the dermatologists who wanted larger volumes of basal cell carcinomas for a specific study. Even in these studies, data for other projects were obtained; for example, the wound seeding study and another example is a beryllium granuloma. On several occasions patients were admitted by other institutes for material for special in vitro studies (mostly thyroid adenomas) and patients with obstructive jaundice in order to obtain bile for biochemical studies. It is difficult from the data available to determine just which cases were admitted for specific studies.

Major Findings:

In supplying service for this project, the surgeons of NCI were required to answer 674 requests for intramural consultation. This compares with 643 for 1959 and 582 for 1958. In addition, the surgeons of NCI assisted extramural consultants with diagnostic and/or therapeutic problems in 410 patients. Surgery was involved in 535 instances (plus assisting outside consultants in 25 additional cases).

An example of this service function applied to animal research is given in the following report of Dr. George Miller regarding his contribution to the "Study of the excretion of methotrexate by the liver in the dog."

A chronic preparation to collect the totally diverted biliary drainage of the dog at will intermittently, is made by inserting a polyethylene tube in the proximal end of the transected common bile duct and a loop is brought out through the flank wall subcutaneously. The distal end of the tubing is re-introduced into the peritoneal cavity and inserted into the distal end of the common bile duct. When collection of the biliary drainage is desired, the subcutaneous loop of tubing is accessable through a small skin incision.

Significance to Cancer Research:

This project is a service function for other branches of the National Cancer Institute and the National Institutes of Health.

Proposed Course of Project:

The number of such cases seems to be increasing as demand for assistance in obtaining tissue for special study increases.

Part B included Yes / / No /X/

Serial No. NCI-10-751(c)
1. Surgery Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Project Title: Evaluation of Cancer Surgery, As To Its Effectiveness and Safety in Providing Definitive Therapeutic and/or Palliative Care for Cancer Patients.

Principal Investigator: Dr. Robert R. Smith, Dr. Alfred Ketcham.

Other Investigators: Dr. Marvin Arons, Dr. Robert Hoye, Dr. John Dillon, Dr. Jack Bloch.

Cooperating Units: None

Part I. The Role of Bacterial Infection in Cancer Surgery. (Dr. Ketcham)

Part II. Results of Surgical Therapy of Cancer of Head and Neck Regions. (Drs. Smith, Arons and Hoye)

Part III. A Study of the Biological Behavior of Carcinoma of the Cervix in Relation to Present Day Therapy. (Dr. Smith, Dr. Dillon)

Man Years (calendar year 1960):
Total: 2½
Professional: 2
Other: ½

Patient Days (calendar year 1960)

Part I. The Role of Bacterial Infection in Cancer Surgery. (Dr. Ketcham, Dr. Bloch)

Methods Employed:

As part of the evaluation of radical surgery, for primarily head and neck and pelvic cancer patients, there was significant evidence that postoperative wound infections were playing a major part in operative morbidity. For this reason a one year retrospective experience of the Surgery Branch was undertaken with particular interest directed at factors interacting between the patients and staphylococci to produce wound infections. Attention was also paid to situations predisposing to such infection following cancer surgery. The records of all patients who underwent surgery and received their total pre- and postoperative care

on the surgical unit during the year 1959 were reviewed. These included 461 operations, 247 of which were major, usually radical en bloc resection of tumor. Survey of the 214 minor procedures in the same period showed only 3 postoperative infections and the study was accordingly limited to major procedures. Postoperative infections were defined as invasion of the tissue by pathogenic organisms in a way that injury followed by reactive phenomena results. The study was limited to postoperative infection of surgical wounds which led to suppuration and some degree of wound breakdown. The 247 patients who underwent major surgery were divided into four categories with respect to their host relations with staphylococci on the basis of preoperative cultures.

1. Asymptomatic Staphylococcus aureus carriers with organisms present in the floor of the nose, sputum, or skin on admission to the hospital (49 cases).

2. Patients with local minor staphylococcal infections in furuncle or skin reactions (19 cases).

3. Patients with staphylococci cultured from exposed necrotic tumors (11 cases).

4. Patients with no staphylococcus aureus in preoperative cultures (93 cases).

5. Patients with no preoperative cultures (75 cases).

All staphylococcus aureus discovered was bacteriophage typed and the bulk of the personnel in active contact with the surgical patients underwent routine nasal cultures several times during the year. Preoperative antibiotic therapy was carried out in only 6 of these cases. The air supply of the operating rooms used for the majority of procedures was entirely separate from the rest of the hospital and served by a hot and cold duct filtered air system with temperature maintained fairly constant between 68° and 70° F. Within the operating rooms a total volume of room air was exchanged every four to five minutes. Air was sampled at random intervals during an eight month period using three sampling methods.

Part Two of this infectious study program was then undertaken after the completion of the above retrospective study. All patients had cultures taken on admission to the unit from the nares, skin at the site of proposed operation, and tumor if visible. The patients were divided into a carrier and a non-carrier grouping depending on the presence of a coagulase positive staphylococci

culture. Through cooperation of the Biometric Branch, a double blind protocol was set up using placebos and chloromycetin, 4 grams daily. Only the Pharmacy Department knew whether the medication was placebo or not and it was made available to us by various routes of administration in an indistinguishable form. The study was subdivided so that there would be equal representation of placebo and drug in both carrier and non-carrier groups. Similarly the different types of surgical procedures were equally divided. Infections occurring during the ten day postoperative study period of antibiotic therapy was carefully recorded. The identity of the agents remained unknown until the results of the study had been tabulated.

Major Findings:

In the retrospective study of the 247 operative cases there were 41 wound infections. Thirty of these infections, or 70% of the total, involved staphylococcus aureus hemolytic coagulases positive either in pure culture or as predominate organisms in a mixed culture. Of the remaining, E. coli was a predominate pathogen in 12%, mixed infection with E. coli and proteus was present in 6%. Proteus alone was found in 6%, and there was mixed infection with E. coli and pseudomonas in 4% and staphylococcus epidermitis occurred only in 2%. Twenty of the 30 wound infections occurred in sites involving ischemic tissue. This ischemia was related either to preoperative tumor radiation or to the radical dissection necessary to the completeness of the operation, as for instance in a radical groin dissection. Three groups of procedures carried a high incidence of infectious morbidity. The head and neck operations in which the hypopharynx or trachea opened into the operative wound in the course of the radical resection of the tumor, emergency laparotomy in which unprepared bowel was opened into the operative field, and radical groin dissection.

Bacterial Factors in Wound Infection: Twenty-four of the 30 patients who developed staphylococcal wound infections were preoperative carriers of staphylococci, 13 asymptomatically, 2 with minor skin infections, and 9 with staphylococci growing in necrotic tumor. There were 5 wound infections with staphylococcus aureus, phage type 80/81. All 5 of these individuals had the identical organisms cultured preoperatively from the tumor in two instances and the sputum and the nose in three instances. There were found wound infections with staphylococcus aureus phage type 83, 3 of which carried the identical phage type organism when they were admitted to the surgical unit. There were 13 wound infections with untypable strains of which 3 occurred in patients with tumor previously infected with an untypable organism, 5 in asymptomatic

nasal carriers of an untypable strain, 2 were non-carriers and 3 in patients who did not have preoperative cultures. There were 8 infections, each with a different specifically identifiable phage type in which the pre- and postoperative phage types were identical, 3 having had infected tumor cultures and 5 from asymptomatic nasal cultures. Table I reviews the correlation between postoperative wound infection and the preoperative host relationship of the patient with regard to staphylococci. Twenty-four of the 30 staphylococcal infections appeared to be auto-infections and each developed a wound infection with the identical organism cultured preoperatively. Nine of these patients had staphylococci in the tumor and subsequent infection may have been inevitable. Fifteen patients carried staphylococci in hidden fashion in their noses or in seemingly insignificant furuncles. In only 2 cases was the infection with an organism not preoperatively cultured from the patient. There were 4 patients who did not have preoperative cultures.

Cross infection between patients or between the active personnel contacting the surgical patients was studied without any significant findings being concluded. The sampling methods in the operating room showed consistent bacterial counts below 10 colonies per cubic foot. When staphylococcus aureus was infrequently isolated in the operating room, the organisms at no time coincided with the bacteriophage types reported in any of the postoperative wound infections but did correlate with phage types present in operating room personnel.

Results of the double blind prophylactic postoperative antibiotic study is shown in Table 2. It is seen that there was a 14% incidence of wound infection in 43 cases who received antibiotics, 28 of which were non-carriers and 15 of which were carriers of pathogenic staphylococci. This compares favorable to the previous years study which showed a 17% incidence of wound infection in all cases. When no antibiotic was given (14 carrier patients and 22 non-carrier patients for a total of 36 patients), there was a 52% incidence of wound infection in the carrier patients, regardless of treatment was 45%, and in the non-carrier regardless of treatment was 24%.

Significance to Cancer Research:

Cancer patients are often debilitated from their primary disease and in turn have a tendency to tolerate surgery less well than the usual individual. The morbidity associated with a postoperative wound infection may be of remarkable significance in carrying out the proposed study protocol for which the patient is

admitted. It is hoped that the results of this study will direct the physician more accurately in his indications for prophylactic antibiotic therapy.

Proposed Course of Project:

The project without the antibiotic study is being continued with routine cultures being taken on admission as well as throughout the hospitalization. We will attempt to correlate the culture results, which will include skin and lymph nodes taken at the time of surgery, with the incidence of wound infection and a thorough evaluation of the incidence of cross contamination among hospital personnel and patients themselves. Tentative plans are underway to undertake a prophylactic pre-surgical antibiotic double blind study which theoretically could lower the 14% incidence of wound infection by allowing the patient a coverage by appropriate antibiotics during the operative procedure itself.

TABLE I

THE CORRELATION OF POSTOPERATIVE WOUND INFECTION WITH PREOPERATIVE STAPHYLOCOCCAL CARRIER STATE

Preoperative Host-Staph. Relationship	No. of Patients	No. of Staph. Wound Infections	No. of Auto Infections	No. of Heterogenous Infections	No. of Non-Staph Infections	No. of Patients without Postop. Infection
Asymptomatic Nasal Carriers	49	13	13	0	4	32
Carriers with Minor Skin Infections	19	2	2	0	0	17
Carriers with Staph. in Necrotic Tumor	11	9	9	0	0	2
Total Carriers	79	24	24	0	4	51
Patients without Preop. Cultures	75	4			2	69
Non-Carriers	93	2	0	2	5	86
Total	247	30	24	2	11	206

TABLE II

PERCENT OF PATIENTS WHO DEVELOPED INFECTION

	On Antibiotics	No Antibiotics	Total
Carrier	20% 3/15	71% 10/14	45% 13/29
Non-Carrier	10% 3/28	40% 9/22	24% 12/50
TOTAL	14% 6/43	52% 19/36	

Part II. Results of Surgical Therapy of Cancer of Head and Neck Regions.

Objectives:

About 1/3 of the patients available for study by the Surgery Branch have cancer of the head and neck region. As this clinical material matures a number of interesting observations are becoming apparent.

The patients with head and neck cancer usually have fairly extensive disease, and the over all survival results are very similar to that reported in the literature. Two interesting studies of this case material have been made by 1) Dr. Marvin Arons on clinical behavior of these patients in reference to local recurrence and distant metastases; and by 2) Dr. Robert Hoye on a clinical pathological study of cases coming to autopsy.

Dr. Arons' study follows.

Objectives:

The objectives of this review of 89 patients with head and neck cancer are five-fold:

1. To stress the incidence of distant metastases occurring during a clinical study.

2. To determine the value and need of preoperative and post-operative chest and bone X-rays in demonstrating distant metastases.

3. To re-emphasize the early appearance and frequency of local recurrence.

4. To correlate cervical lymph node metastases with local recurrence and distant metastases.

5. To illustrate the use of life-table analysis of head and neck cancer data.

Methods Employed:

The 89 patients in this study were admitted to the Surgery Branch of the National Cancer Institute between July, 1953 and January, 1960. None was lost to follow-up which closed on August 1, 1960, giving a minimum follow-up period of 6 months and a maximum of 76 months. All cases had extensive invasive epidermoid

carcinoma except for two with adenocarcinoma of the paranasol sinuses. Included in the project were all consecutive admissions with cancer of the paranasal sinuses (13), tongue (12), larynx (12), floor of mouth (11), tonsil-pharynx (10), pyriform sinus (8), multiple intra-oral (6), alveolar ridge (5), buccal mucosa (5), buccogingiva (4), and palate (3).

Major Findings:

As determined by life-table analysis of the 77 cases treated by definitive surgery, the cumulative percent with distant metastases was 23% at 2 years, and the cumulative percent with local recurrence was 47% at 3 years. Median time interval from surgery to local recurrence was 5 months with 71% recurring by 8 months and 100% by 3 years. Postoperatively, 60% of distant metastases developed by 8 months, and all but 1 case by 2 years. No patient had radiologic evidence of pulmonary or skeletal metastases on admission. Since 50% of those with postoperative pulmonary or skeletal metastases had symptoms referable to those systems, only 1 in 10 of all patients followed after definitive surgery would have benefitted from routine metastatic surveys. No statistical correlation could be found between cervical lymph node metastases and local recurrence or distant metastases in this small series.

Dr. Hoye's study was designed to demonstrate the incidence of metastases below the clavicle in patients coming to autopsy after therapy for cancer of the head and neck region.

Dr. George Crile, Sr., originally reported a less than 1% incidence of metastases below the clavicle in head and neck cancer. More recent reports state that the incidence is usually between 5-25%.

The impression at the Clinical Center has developed that distant metastases were being seen more frequently and that the jugular vein was rarely involved with tumor.

The clinical records of 42 consecutive patients dying of epidermoid cancer of the upper respiratory and alimentary tract, and coming to autopsy were reviewed.

Major Findings:

In the 42 cases coming to autopsy, 23 (56%) demonstrated metastases below the clavicle, and in 19, none could be found. The brain was involved in 9. This was considered a direct extension of the primary tumor and not a metastases as defined. Forty-five per-

cent of patients had disease limited to a potentially curable site. The patient most likely to develop distant spread of his disease, presents with a large primary tumor, early cervical lymph node involvement, has 1 or 2 local recurrences treated after primary treatment of his tumor and lives an average of 16 months after primary treatment. Cervical lymph node involvement usually precedes distant spread, but internal jugular vein involvement is seldom seen. Twenty-two of the 23 patients with distant metastases had lung involvement at autopsy. In 11 of these patients, there was clinical evidence of distant metastases before death, (7 with pulmonary involvement).

The main problem during life in this group of 42 patients was control of the primary tumor and its regional metastases.

It is interesting to note that the 2 studies complement each other, in that although distant dissemination of head and neck cancer is not as common as seen with many other cancers, it can be demonstrated during the life of 20-25% of patients, and at autopsy is over 50%.

Significance to Cancer Research:

With a prolongation of life by more extensive surgery and efficient irradiation, the likelihood of developing distant metastases probably increases. It is of clinical significance to report the incidence of distant metastases in living patients as well as in cases selected by autopsy. From the data presented, the concept that cancers of the head and neck seldom spread below the level of the clavicle should be abandoned. When cancer spreads to the cervical nodes, no definite correlation with local wound recurrence or distant metastases could be found. Perhaps thinking will change about the role of cervical nodes as an effective barrier to further dissemination of head and neck cancer.

Both of these studies should lead to continued study to develop means of controlling cancer at its primary site.

Part III. A Study of the Biological Behavior of Carcinoma of the Cervix in Relation to Present Day Therapy.
(Dr. Smith, Dr. Dillon)

Objectives:

For the past 30-40 years, carcinoma of the cervix was largely treated by irradiation. The clinical course of cases treated by this modality has been fairly well documented. The over all survival rate has increased from ±20 to over 45% and patients who were not "cured," died usually of complication of local pelvic recurrent cancer. Most autopsy studies show only about 20% of such cases having cancer above the pelvis.

During the past 10-15 years, in selected centers, attempts are under way to surgically excise cervix cancer; either by radical hysterectomy or by pelvic exenteration. This mode of therapy has been under study at NCI and forms the basis of this report. The study has attempted to show that radical surgery does offer increasing chance for "cure," even in late stages of the disease. It has been observed, also, that changes in mode of therapy apparently changes the behavior of the cancer. This study was undertaken to demonstrate this phenomenon.

Methods Employed:

This study was a chart review of the 131 patients with carcinoma of the cervix, admitted to the Clinical Center between August 1953 and January 1, 1959. Twelve of the 131 were excluded from the study for, 1) in 6 instances diagnosis was insitu-carcinoma, and 2) in the other 6 the patients were referred here after previous treatment for invasive cervix cancer, but did not have carcinoma on admission here.

The charts were reviewed, and data tabulated as to factors in past history, stage of disease, complications of the disease and therapy, type of therapy and behavior of the cancer as manifest in follow-up studies.

Major Findings:

All patients had histologically proven carcinoma of the uterine cervix; 117 were epidermoid, 2 adenocarcinoma and 1 adenosquamous carcinoma.

Extent of Disease on Admission Here: 50% of the patients had

had prior therapy before being seen here. The League of Nations classification was as follows:

Stage	1	Carcinoma Cervix	3	patients
"	2	" "	12	"
"	3	" "	5	"
"	4	" "	38	"
Postradiation (unclassifiable)			62	
		TOTAL	120	

In 29 patients, clinical studies demonstrated inoperable spread of the cancer. Metastatic spread was proven shortly after admission in 6 cases by biopsy of a metastatic supraclavicular node. Five others were admitted in uremia and died shortly after admission. In 11 of the remaining 19 cases, metastases were demonstrated beyond the pelvis and surgery decided aginst, and in 8 cases the tumor was considered inoperable because of extensive local tumor.

Summary:

29	patients (24%)	not operable on admission.
31	" (26%)	explored and found inoperable.
17	" (14%)	had a Radical Hysterectomy and node dissection.
8	" (7%)	had an anterior exenteration.
35	" (30%)	had a total pelvic exenteration.

The 60 patients upon whom definitive surgery was performed had their operative specimen histologically examined by Surgical Pathology Department and classified according to the Meigs-Brunschwig classification with the following results:

Stage	A	3	specimens
"	B	6	"
"	C	13	"
"	D	6	"
"	E	28	"
"	F	4	"
	TOTAL	60	

It is thus apparent that only a small number of our patients presented with disease limited to the cervix.

Vital Statistics: 45% of 120 patients in the study were negro and 55% Caucasian. No other races were represented. The mean age of the group was 48.5 years. The oldest patient under-

going exenteration was 74 years old and the youngest 32. Four patients were from outside the Washington, D.C. area.

Preoperative clinical findings: The mean time from diagnosis to admission was 7.3 months. This figure dropped to 3.8 months in the group that was resectable, and 10.7 in the non-resectable ones. The presence of lymphatic invasion (node metastases), in the surgical specimens did not seem related to the time it took patients to receive definitive therapy.

Anemia: A significant number were anemic on admission, only 33 of the 120 had a hemoglobin of 12 gm. or greater; 71 of the patients had a reversed A/G ratio. Fifteen of 19 patients with normal hemoglobin and A/G ratio were resectable, while only 45% of those with abnormal values were resectable.

Pain: It has been our impression that 2 factors implied a grave prognosis, edema of the lower extremity and radiating pain down a leg. Fifty-nine of the group complained of some kind of pain on admission here, 12 of these had radiating leg pain. Only 2 of the 12 were operable, and both died with recurrent cancer.

There were 14 patients admitted with edema of the lower extremity, only 3 of these were resectable, and none of them remained free of cancer.

Extent of tumor as demonstrated preoperatively: It has been known for some time that physical examination was of limited value in estimating the extent of cancer present in the pelvis. Our experience bears this out. Postradiation recurrent pelvic cancer is especially difficult to evaluate. Of 30 patients with palpable involvement into the lateral parametria by pelvic examination, only 21 had any tumor growing laterally. Palpable extent of disease can rarely be used as an absolute criteria of inoperability.

The surgeon's ability to evaluate anterior and posterior extension (i.e., into bladder, and/or rectum) is also not too reliable. The surgeon was only 50% accurate when he described a bladder involved by palpation; or when cystoscopic examination was suggestive. When the cystoscopist described bladder invasion, he was always correct. Clinical evaluation was significant in one aspect, those patients in which the bladder was thought to be free of tumor had a higher resection rate (63%) than those with clinical evidence of bladder invasion (44%). However, the recurrence rate for the 2 groups was essentially the same.

Intravenous pyelograms were found to be a fairly reliable prognostic sign as shown by the following Table.

	No. Pts.	No. Pts. not Explored	No. Pts. Explored	No. Pts. had Hysterectomy	No. Pts. had Exenteration	No. Pts. Free of Cancer
I.V.P. Normal	59	9	10	14	26	21
I.V.P. Abnormal but without Obstruction	7	0	2	3	0	1
Unilateral Obstruction	35	8	13	0	14	6
Bilateral Obstruction	18	9	6	0	0	0
Not Done	1	1	0	0	0	0
TOTAL	120	27	31	17	43	30

When unilateral obstruction was observed, it was always found to be due to carcinoma. The only exception to this rule, is one instance in which radiation fibrosis, associated with an impacted stone, produced obstruction.

On the other hand, a normal I.V.P. does not necessarily rule out invasion of a ureter by carcinoma (1/4 of this group had negative I.V.P.).

Preoperative Complications: During the preoperative work-up the following other complicating diseases were found:

Hypertension, and other Cardiac Diseases	20
Pulmonary Disease	2
Liver Disease	13
Endocrinopathies	5
Gastrointestinal Disease	2
Gallbladder Disease	3
Center Nervous System Disease	2
Obesity	24
Other	23

It should be noted that preoperative complications did not seem to be related to those occurring during the postoperative period, nor did those with complications of any sort have a higher rate of operative or postoperative complications.

Prior Treatment:

58 patients had no definitive treatment prior to admission.
49 " had irradiation therapy.
5 " had prior surgery.
8 " had radiation and surgery.

Only 3 of the 13 that had prior surgery were operable, and none of these survived. The 57 that had radiotherapy had less chance for resection, less bladder invasion and the longer time interval between radiation and surgery, the less chance there was for helping the patient with definitive surgery. The survival of patients that had prior radiation was 39%, and for those that had no prior treatment 46%.

Surgery Performed: All patients in whom it was not possible to demonstrate metastatic tumor beyond the pelvis were explored. The only exception to this was one patient with decompensated mental disease. As noted above, 24% of the patients admitted were found inoperable on clinical grounds, the remaining 76% were explored. Of the 91 explored, resection of the tumor was carried out in 60 (66%), exenterative surgery was found necessary in all but 17. Exenterative surgery requires sacrifice of either bladder, or bladder and rectum.

When excisional surgery was performed, it was associated with a lymph node dissection, and required a form of urinary diversion. For the first 4-5 years of the study, the urinary diversion was accomplished by means of a ureteral sigmoidostomy with either a "wet" colostomy or an isolated sigmoid loop. In the past few years, it has been found more satisfactory to use a segment of ileum as the urinary conduit with a right lower quadrant ileostomy. Since adopting this method of urinary diversion, the nursing problems associated with care of these patients have largely disappeared. In addition, evidence of urinary infection has diminished and we have lost no patients to kidney failure.

Postoperative Complications: Operative mortality for the 60 patients upon whom excisional surgery was performed was 2.3%. (There was no operative mortality in the 31 explored and found inoperable, nor in the 17 radical hysterectomies.) This exceptionally low value is not a true one for at the time of writing this

report, and including over 70 exenterations, the operative mortality has risen to 7%, a value still well below that given in other series. The only true operative deaths in the first 60 radical excisions resulted from intestional obstruction requiring re-exploration 8 days later, with death from operative shock.

In addition, there were 4 patients that died 2-4 months after total pelvic exenteration, usually with uncontrolled cancer and debility. Immediate operative complications included 8 cases of thrombophlebitis, 2 gastrointestinal fistulae and 1 urinary fistula in the exenteration group. There were 2 urinary fistulae in the 17 hysterectomies.

Over 1/2 of the patients undergoing radical surgery in this study required additional major surgery during their period of follow-up study. These included cholecystectomy for obstructive jaundice, resection of lung metastases, relief of intestinal obstruction from recurrent cancer, etc.

Urinary complications have a primary problem associated with radical pelvic surgery. The problems have principally revolved around ascending renal infection with or without obstruction of ureters, renal failure and hyperchloremic acidosis. Previous reports have given our experience with sigmoid diversions by means of wet colostomy, and will not be repeated here. A more complete review of ileal bladder cases will be presented elsewhere.

In 7 patients, renal failure was a primary cause of death, but only one of these died without cancer. In 2 others, terminal uremia was present but not cause of death. In this series, a renal death was associated with recurrent cancer.

Follow-up: At the close of this study in May, 1960 the 60 radical resections are as follows:

17 Hysterectomies ----- (9 living and free of tumor 12 to 63 months.
8 died - 3 free of cancer
5 died of cancer.)

43 Exenterations ------ (17 living 15 to 66 months.
26 died - 6 of these free of cancer.
20 died of cancer.)

There are only 2 hysterectomies and 2 exenterations living and well over 5 years. It must be remembered, however, that most of these cases have been done within the past 5 years, and life

table analysis of survival data for these special groups is not available for study.

Of particular interest in this study was the pattern of recurrent cancer. In 7 hysterectomies with cancer postoperatively 6 (86%) had local recurrence and only 2 (28%) had distant metastases. In 24 exenterations that developed recurrent cancer, 12 (50%) had local recurrence, and 18 (75%) had distant metastases.

This may represent poor selection of cases for more conservative surgery, but it actually represents a fear of the surgeon to sacrifice bladder unless there is physical evidence of bladder invasion. It probably means that the surgeon should do the more radical exenteration in some cases selected for hysterectomy and, (with present inability to control growth of metastatic cancer) not do radical surgery on cases with clinically extensive disease. The exenteration group had a median time from operation to death 6 months longer than the hysterectomy group, possibly allowing a longer time for metastases to occur. Recurrent local cancer or metastases developing after 18 months was rare. Of 31 cases with recurrent cancer, only 6 were found after 18 months, and only 2 after 36 months. It would seem that a fair degree of assurance can be given patients after a 3 year survival.

It must be remembered, that in such a study as this, a more accurate measure of success of a procedure is the help it affords a patient. The measure of palliation afforded is impossible to evaluate. It can be said, however, that during the period of this study, this procedure has been accepted by our "medical community," and all of the Gynecological Departments of the local medical schools send us cases for study. We continue to receive referrals from practicing physicians from as far away as Florida, and find that a patient returned to the referring physician after an exenteration most frequently is followed by additional referrals from that same physician.

Proposed Course of Study:

This group of cases provides material for a number of studies other than the mere statistical study of survival. The inoperable group provided material for the study to show the effect of deficiency states on spread of cancer. The urinary diversion necessitated by the pelvic exenteration requires study of problems of postoperative infections.

The study will continue and probably will require several more years before the end results can be determined.

Significance to Cancer Research:

A study of carcinoma of the cervix is underway at several large centers where radical surgery is being used. The value of this study lies not so much in the efficacy of radical surgery but in the fact that our patients have been worked up preoperatively and pathologically and followed in great detail so that the behaviour of carcinoma of the cervix and alterations in the biological behaviour associated with radical surgery can be studied. In addition, the ileal bladder is an important adjunct to the ablative procedures employed in cancer surgery. Its rationale lies in the necessity of providing a replacement for an organ removed because of involvement by tumor.

Part B included Yes /XX/ No /__/

NCI-10-751(c)
Serial No.

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Romsdahl, Marvin M, Stanley, Harold R., and Smith, Robert R.: Fibrous Dysplasia (Ossifying Fibroma) of the Maxilla in a Child--Case Report and Discussion. Surgery 48:6, 1107-1115, 1960.

Smith, Robert R.: Panel Discussion on "Present Status of Investigation in Surgical Adjuvant Chemotherapy." NCI Monograph No. 3 page 121-125, Aug. 1960, Conference on Experimental Clinical Cancer Chemotherapy.

Serial No. NCI-9-752(c)
1. Surgery Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Part I. The Virus Treatment of Human Carcinoma of the Cervix.

Part II. Pyridoxine Deficiency in Conjunction with Virus Treatment of Cervix Cancer.

Principal Investigator: Part I. Dr. Robert R. Smith, Dr. Robert J. Huebner, Dr. Robert B. Couch, Dr. Robert A. Manaker.

Part II. Dr. Robert R. Smith, Dr. Robert B. Couch.

Other Investigators: Part II. Dr. Edward McLaughlin, Dr. Robert A. Manaker, Dr. Helen Dyer.

Cooperating Units: Part I. Laboratory of Biology, NCI; Laboratory of Pathology, NCI; and Laboratory of Infectious Diseases, NIAID.

Man Years (calendar year 1960):
Total: 1
Professional: 3/4
Other: ¼

Patient Days (calendar year 1960)

Part I.

Objectives:

In the introduction of viruses associated with human tumors project, the theoretical associations that may occur between viruses and tumors are presented. A cooperative project between the laboratories listed above has been underway for 6 years. This project involves the study of the effects of artificially infecting human tumors with viruses. Previous animal reports have presented the yearly progress and the publications resulting. Studies with the adenoviruses with and without concomitant administration of cortisone resulted in encouraging therapeutic responses. In an effort to enhance the resultant oncolysis, a serial study was

undertaken in a clinical situation utilizing Coxsackie B3 virus. This study is based on the demonstration by Suskind, et al, that passage of virus tumor to tumor in a rat tumor system resulted in enhanced oncolytic effect. This report will cover study of efforts to make Coxsackie B3 virus more oncolytic by patient to patient passage.

Methods Employed:

Part I.

Two methods of treatment and study have been employed. The first method consisted of a 14 day isolation protocol. The virus was injected directly intra-tumor on day 1. Virus cultures were made of vaginal discharge and tumor biopsies on days 0, 2, 4, 6, 8, 10, and 14. Serum was collected simultaneously for antibody titers. Observation for tumor necrosis was made at the time of culture. Temperature, pulse, and respiration were taken every 4 hours and no antipyretics were used except for temperatures greater than 40° C. Daily observation was made for clinical symptomatology. The second method involved a 6 day protocol. The same methods were used except for the fact that on day 6 approximately 5 grams of tissue was removed for virus isolation and the study period terminated.

1. Methods of virus isolation: Swabs of vaginal discharge were immersed in 2 cc. of salt solution. This material was inoculated on HeLa tissue cultures and observation made daily for cytopathogenic effect of Coxsackie type. Cervical biopsies were homogenized and this material was handled in a manner similar to the swab cultures. The 5 grams of tissue removed was sectioned. Half the material was used for Maitland culture and the other half homogenized for isolation as above. The harvested Maitland fluid was also inoculated on HeLa cells.

2. Preparation of injectable material: Pools of injectable material were prepared by inoculation of a 32 oz. HeLa bottle with the original material of one of the isolates. The harvested bottle fluid was used as a pool. The other source of injectable material was the filtered Maitland and homogenized fluid.

3. Safety tests: All material used for human injection received animal testing and tissue culture identification with specific antiserum prior to human use.

4. Antibody titers: Serum antibody titers to Coxsackie B3 virus were determined by neutralization in tissue culture using a 1:4 dilution of standard and unknown serum.

Patient Material

All patients in both parts of this project had epidermoid carcinoma of the cervix and were under the care of the Surgery Branch, NCI. In most instances these patients were under study for other projects, usually inoperable, or postradiation recurrent tumor was present. A few received virus therapy as a preoperative measure.

Major Findings:

Part I.

Twenty-seven patients received from 2 to 20 cc. of virus solution, intra-tumor. The Coxsackie B3 virus (originally isolated by Dr. Huebner from 9 cases of epidemic pleurodynia and passed through 12 rat HeLa passages) was in a titer of 10^5 - 10^8. The quantity and titer of virus used bore no relationship to results obtained.

It is pertinent to mention that no evidence of Coxsackie infection occurred among other patients or doctors or nurses attending on the ward. All doctors and nurses who failed to exhibit antibodies to Coxsackie B3 showed no change in antibody status on recheck one year later. The following is a summary of pertinent data from this study.

Virus Recovery: For 4 days following injection, attempts to recover virus was successful in all cases. Between 4-8 days the frequency of recovery dropped rather precipitously. Virus was recovered in only 3 patients after day 8, and in only one after day 11.

Titer of virus in vaginal discharge and tumor tissue revealed that associated with a decline in quantity of virus recovery, there was a decline in quantity of virus recovered. However, when all specimens from a patient were totaled, the quantity of virus recovered was greater than that administered. This is considered evidence of intracellular virus multiplication. Recovery of virus from 6 immune patients was accomplished for 5 days following injection in one patient, and for 2 days in another. In the remaining 4 patients no virus was recovered, demonstrating that virus survival in the cancer-immune host is infrequent.

Swab culture of throat and anus were positive intermittently. No throat culture was positive after day 6, and no anus culture after day 8. Only one positive blood culture was obtained and this

was on day 2. At this time the patient was asymptomatic and afebrile. All blood cultures taken at the time of fever or symptom were negative.

Tissues taken at postmortem examinations were all negative for virus, however, these were all many days after virus inoculation.

Serum Antibody Titers: All patients demonstrated a rise in titer following inoculation. The rise began as early as the 4th day, and by day 8, all patients had a titer of greater than 4. The very early rise seen in some patients suggests the anamnestic response indicating previous immunity had been present at some time. Patients with a pre-injection antibody titer of greater than 4, demonstrated a rapid rise in titer. Absolute titers 10-14 days post-injection varied between 256 and 2048.

It was noted that increase in antibody titer seemed to be associated with decreasing recovery of virus.

Clinical Findings: Although Coxsackie B_3 is known to produce rather severe pleurodynia symptoms, asceptic meningitis, myocarditis of the newborn, etc., careful observations failed to reveal significant clinical symptoms, except for an occasional very mild low pleuritic type of "soreness". 18 patients demonstrated mild fever. In fact, symptoms and signs of a clinical virus infection were conspicuous by their absence.

It was noted that 10 patients who received Bertha adeno virus alone did not develop clinical evidence of illness. However, when both adeno virus and Coxsackie B_3 were given in combination within 12 hours temperature would rise to 39° - 40° C. and undergo slow defervescence. Associated with this febrile reaction was general malaise similar to that seen with influenza. All blood cultures from these patients were negative for virus.

Degree of Tumor Oncolysis:

In general, Coxsackie B_3 produced less necrosis of tumor than that seen with adeno virus. A 3 or 4 plus reaction was seen in only 3 instances (and 2 of these were associated with B_6 deficiency).

Serial passage through 12 patients did not produce a clear cut change in its oncolytic property. The last 3 patients receiving material from 12th passage, experienced a 0. 1+ and 1+ reaction.

In view of the many factors that can alter the degree of oncolysis, it is impossible to state that the human passage experience will, or will not, be similar to that found in the animal model.

The experience with Coxsackie virus was similar to that with the adeno virus in regard to the hemostatic effect. In several instances severe vaginal bleeding was stopped after local injection of virus into the tumor.

Proposed Course of Project:

In view of the suggestion observed in the last few patients that passage of virus through human cancer may result in enhanced oncolytic properties of the virus, it is believed advisable to continue the study for a few more passages.

Part II Pyridoxine Deficiency in Conjuction with Virus Treatment of Cervix Cancer.

Objectives:

In the virus treatment of cancer there is an apparent relationship between frequency of antibody formation and frequency of recovery of virus from the injected tumor. It is not known to what extent serum antibody, as opposed to other mechanism of host resistance to infections, influences virus survival. However, it was felt that if antibody formation could be altered, then perhaps virus survival would be prolonged and degree of tumor oncolysis be increased.

Previous study has shown that antibody formation can be altered by deficiency of vitamin B_6 and that a potent antagonist to B_6 was available.

Methods Employed:

Two protocols were designed to study the effect of pyridoxine deficiency on antibody formation, virus survival, and tumor oncolysis in the human host. Studies were included to clarify the nature of human pyridoxine deficiency and measure the degree of deficiency state. This study is in progress, and this constitutes an interim report.

Part II.

Design of protocol:

1. Base line (5-7 days) - Clinical observations and routine studies. Diet - normal.

2. Control Period (12 days) - Blood studies, urine studies, weight records, intake and output records. Diet - B_6 deficiency supplemented with 5 mg. of B_6 daily.

3. Deficiency Period (variable) - Diet - B_6 deficiency + desoxypyridoxine.

4. Deficiency Period (with virus) - Diet - B_6 deficiency + desoxypyridoxine.

5. Recovery Period I (variable) - Diet - B_6 deficiency + desoxypyridoxine + pyridoxine.

6. Recovery Period II (variable) - Diet - B_6 deficiency + pyridoxine.

7. Control - Normal diet.

Diet: 35 calories per kilogram weight.

Vitamins - Compound #102
Vitamin D - 800 u
Vitamin A - 5000 i.u.

Minerals - NaCl, 5 grams

Protein - 125 grams of vitamin free casein

Carbohydrate - Dextrose q.s. to make 2/3 of remaining calories

Fat - Corn oil q.s. to make 1/3 of remaining calories.

The diet is mixed in a Waring blendor with distilled water and distributed into 6 equal feedings which are taken over a 12 hour period. Distilled water is provided ad lib. Food flavoring is added to the diet. No other intake and no lotions or creams that might be vitamin containing are used.

Clinical observations: Daily clinical checks are made to ascertain any pertinent symptoms or physical findings that may be pertinent to the study. The patients were weighed daily and accurate intake and output records were kept.

Routine laboratory studies: A routine urinalysis, BUN, FBS, creatinine, uric acid, cholesterol, TSP A/G, Ca, P, Na, K, Cl, CO_2, alkaline phosphatase, BSP and Transaminase, Hgb, Hct, WBC with differential, RBC, and indices were performed at least once weekly by standard laboratory methods.

Special studies relating to B_6 deficiency: Whole blood transaminase studies were done twice weekly in one patient. Bone marrow aspiration was performed before and during deficiency in one patient. Xanthrenic acid excretion in the urine was quantitated after a load test of Tryptophane several times in each patient. This compound accumulates in B_6 deficiency. Other studies performed on some of the patients included creatinine clearance, PSB excretion, and Fishberg urine concentration tests. Electroencephalograms and electrocardiograms were done when indicated.

Studies relating to tumor, virus, and host response to infection: Typhoid-paratyphoid vaccine, blood group substance, and Coxsackie B_3 virus were used as antigens and were given to the patient at a time determined by clinical and laboratory state. Serum was drawn 12-14 days later for antibody titer. Antibody against typhoid and paratyphoid was measured by slide agglutination, blood group substance by hemolysis of red cells, and virus antibody by neutralization in tissue culture. Virus cultures were done according to the previously described protocol for virus treatment of cancer. The cellular response of the host to an inflammatory stimulus is studied by the Rebuck technique which consisted of scraping skin down to the corium, applying a drop of Diptheria toxin, and covering the area with a slide. Differentials of cellular exudate are studied at 3, 6, 12, and 24 hours following inoculation. This procedure is performed before, during, and after the deficiency period. Tumor oncolysis is graded according to the previously described scale.

Part II.

Major Findings:

Five patients with carcinoma cervix have been through this protocol. Three of them received 200 mg. of desoxypyridoxine per day, one 50 mg and one 100 mg of the antagonists per day.

One of the patients receiving 200 mg of desoxypyridoxine died of septicemia without developing evidence of vitamin deficiency. She had bilateral ureteral obstruction and secondary ascending pyelonephritis. All of the other patients developed classical signs of deficiency. The patient that received 100 mg. of the antagonist is still living one year after her therapy. She developed convulsions secondary to the vitamin deficiency. The seizures were easily controlled with vitamin B_6.

Summary:

Resistance to infection: Of the three modalities of resistance to infection studied, namely antibody formation, cellular migration to an inflammatory stimulus, and peripheral leukocyte counts and differentials, only one has shown a suggestion of being influenced by B_6 deficiency. This modality is cellular migration to an inflammatory stimulus. The 24 hour mononuclear response, particularly with high doses of desoxypyridoxine, is markedly impaired. There is a suggestion that antibody formation is delayed and absolute titers low. More cases may statistically prove this to be so. Virus seems to survive longer in the tumor than usual and degree of oncolysis in two cases was greater than that in 28 cases without associated B_6 deficiency.

Previously reported findings in human pyridoxine deficiency: The mental symptoms are different in that mood swings with periods of stability and orientation are more frequent than those previously reported. The skin changes in J.J. were far more severe than any reported. Only two patients exhibited a marked lymphopenia. Other blood changes could not be studied in these patients as all were bleeding during study. The markedly elevated xanthrenic acid excretion in patient J.J. was much more than that reported by other authors. It is felt by this observer that the changes seen were consistent with those previously reported, but in certain instances were more severe than any reported.

Unreported findings: The markedly impaired renal function and the profound catabolic responses are heretofore unreported. The explanation and significance of these findings is unknown at present. The elevated fasting blood sugars are also unreported, but may be explained by the known toxicity of xanthrenic acid for the B cells of the islets of Langerhans.

The oncolytic effect of the virus in the 3 cases where observations were valid showed a 3 or 4 + response in all cases. This clinical observation was very impressive and the prolonged "remission" observed in case #5 has been very rewarding. This

patient had "terminal" extension of her cervix cancer surrounding the iliac vessels proven by biopsy obtained at laparotomy. One year later she still has her cancer. It may be growing slowly, but leg edema has subsided, and she is clinically free of disabling symptoms.

Proposed Course of Project:

Part I.

In order to further the encouraging effects obtained thus far in this study, it seems imperative that we know if the virus enters the cell and cell death results. There are techniques available that can provide this answer. If this is true, and all indirect evidence suggests that it is, then one must know what the limitation is on degree of oncolysis. If it is a matter of virus contact with all cells, then all possible routes of infection must be used simultaneously. If it is host resistance to infection, then this must be altered sufficiently to produce the desired oncolysis without detriment to the host. These are the planned approaches, and it is felt that this can be done in the virus passage situation. Ultimately, the specific biochemical upset of the cell as a result of virus infection will be sought.

Significance to Cancer Research:

Part I.

Exploration of new avenues of treatment of cancer in the human is a necessity. New information is being gained on the nature of virus infection in the human.

Part II.

This study demonstrates a means by which a treatment of human cancer can be enhanced. Studies on mechanisms of host immunity may aid in the understanding of human immunity to cancer. The expansion of knowledge on human physiology and biochemistry will aid in the understanding of many neoplastic and other diseases which affect man.

Two more human cases at lower desoxypyridoxine dosage are planned. Further study on the renal function and catabolic abnormalities are planned on laboratory animals.

Part B included: Yes /___/ No /XX/

Serial No. NCI-8-753 (c)
1. Surgery Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Project Title:

Study of Tumor Emboli in Relation to Metastases in Human Cancer.

Part I. Determination of the Frequency and Significance of the Finding of Tumor Cells in Washings Obtained from Operative Wounds Following Definitive Surgery for Cancer.

Part II. Determination of the Frequency and Significance of the Finding of Tumor Cells in Washings Obtained from Peripheral Vein or Veins Draining a Cancer.

Part III. Determinations of Frequency and Significance of Finding Tumor Cells in Postoperative Wound Drainage.

Serial No. NCI-8-753 (c)
1. Surgery Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Titles: Part I

(A) Diurnal Patterns of Occurrence of Circulating Tumor Cells in Blood.

(B) Effect of Endocrine Therapy on Occurrence of Tumor Cells in Blood in Patients with Endocrine-Sensitive Tumors.

(C) Effect of Events in the Operating Room on the Occurrence of Tumor Cells in Blood.

(D) Evaluation of Methods of Study of Frequency and Significance of Tumor Cells in Blood of Hospitalized Cancer Patients.

(E) The Dextran Modification of Millipore Technique for Recovering Cancer Cells from Blood.

(F) A Clinical Study of Circulating Tumor Cells in Malignant Melanomas.

(G) Prognostic Factor of Tumor Cells in Blood.

Principal Investigators: Dr. John T. West and Dr. Richard Malmgren

Other Investigators: Dr. Robert R. Smith, Dr. Ian Spence, Dr. Marvin Romsdahl, Dr. John Potter, and Mrs. Roxane Hume

Cooperating Units: Cytodiagnostic Service, Pathologic Anatomy Branch, NCI, and Tumor Service, USPHS Hospital, Baltimore, Maryland.

Man Years (calendar year 1960):
Total: 7½
Professional: 3
Other: 4½

Patient Days (calendar year 1960):

NCI-8-753(c)
Serial No.

Project Description:

Part I. (A) Diurnal Patterns of Occurrence of Circulating Tumor Cells in Blood. (Dr. West)

Objectives:

This is a clinical study intended to determine diurnal patterns of occurrence of cancer cells in the peripheral blood of patients with various types and stages of malignant disease.

Methods Employed:

Employing venous catheters, blood specimens are collected at hourly intervals for periods of 24 or more hours. The blood specimens so collected are processed by the method of Malmgren and Potter, recovering and staining blood-borne cancer cells on Millipore filters. Filters are screened by cytological technicians and final readings are made by pathologists of the Cytodiagnostic Unit of Pathological Anatomy Branch. The results are recorded quantitatively and plotted against the time of collection, noting any possible relationship to other events during the time of collection.

Patient Material:

Subjects for this study are selected from cancer patients hospitalized at the National Cancer Institute.

Major Findings:

This study has just been initiated and as yet no results are available for tabulation.

Significance to Cancer Research:

A study of this type is necessary to serve as a base line for any clinical studies which will concern the significance of circulating tumor cells in the blood. If one is to attach any significance to the finding of cancer cells in the blood stream based on samples collected at random or at the time of a specific event it is necessary first to know whether cancer cells are ordinarily shed into the blood stream at a nearly constant rate or, if not at a constant rate, if they are shed in some predictable pattern. If one should demonstrate that there is an unpredictable pattern of marked variation in numbers of cancer cells found in the blood stream at different times of the day or night, one would then not be able to give credence to comparison of quantitative results between different patients or between one patient at different times and one would not be able to attribute variation in number of cells found in the operating room to specific events occurring there. Such

comparisons have been made in the past. This has been done without first establishing if there are any basic patterns that would support or contradict such use of these results. It is also possible that if some predictable pattern of variation were demonstrated that there might be implications affecting the time for most advantageous administration of chemotherapeutic agents. Administering such agents into the blood stream at a time when cancer cells in the blood stream are at a peak might enhance the effectiveness of the chemotherapeutic agent.

Proposed Course of Project:

It is intended that intensive studies of several patients representing a variety of cancers will be studied by these methods to determine if there is ever any pattern to the appearance of cancer cells in the blood stream and if there is any variation in this pattern for different types and stages of cancer.

Part I. (B) Effect of Endocrine Therapy on Occurrence of Tumor Cells in Blood of Patients with Endocrine-Sensitive Tumors. (Dr. West)

Objectives:

To determine if varying forms of endocrine therapy (e.g. castration, adrenalectomy, or administration of specific hormones) will affect the presence and number of cancer cells in the blood when the malignancy involved is one of the endocrine-sensitive tumors such as breast cancer, or carcinoma of the prostate.

Methods Employed:

Using the cytodiagnostic methods referred to in Part I, (A), quantitative results of recovery and identification of circulating tumor cells in the blood will be noted and related to the type of endocrine therapy given and will also be related to the other parameters of the clinical progress of the disease.

Patient Material:

Patients will be selected from those NCI hospitalized patients who have endocrine-sensitive cancer.

Major Findings:

This project has just been initiated and results from the first two patients so studied have not yet been tabulated.

Significance to Cancer Research:

The finding of cancer cells in the blood stream has generally

been regarded as a factor of poor prognostic significance. Neither this assumption nor the converse of this assumption has been generally or firmly supported by observation. The possibility that occurrence of tumor cells in the blood stream might indicate that the host is doing well and the tumor not so well usually has not been considered. Should the number of circulating cancer cells increase while all other clinical and radiographic evidence indicate shrinkage or disappearance of primary or metastatic cancer there would be good reason to reconsider whether the numbers of cancer cells in the blood stream is an indication of the progress of the disease.

Proposed Course of Project:

Additional patients with endocrine-sensitive cancers will be studied and quantitative appraisal of circulating tumor cells will be related to the type of endocrine therapy and to various clinical indications of response to the endocrine therapy.

Part I. (C) Effects of Events in the Operating Room on the Occurrence of Tumor Cells in Blood. (Drs. West & Smith)

Objectives:

To study further the relationship of events in the operating room to the numbers of circulating tumor cells found in the general peripheral circulation and in the veins directly draining the cancer. This will be a continuation of studies previously carried out here under the direction of Dr. John F. Potter.

Methods Employed:

Periodic samples of peripheral venous blood, as well as blood from veins directly draining the cancer, will be collected in the operating room in relationship to specific events occurring there, such as induction of anesthesia preparation of skin overlying a tumor, manipulation of the tumor and ligation of vessels supplying and draining the tumor. These samples will be studied by the cytodiagnostic techniques mentioned in Part I, (A), and the results recorded quantitatively.

Patient Material:

Such studies will be carried out on National Cancer Institute patients undergoing attempted extirpation of cancer or during diagnostic procedures such as curettage of endometrium, retrograde pyelograms and prostatic massage.

Major Findings:

The number of such studies concluded to date are not sufficient

to permit significant tabulation and analysis of the result. However, from a limited number of such studies, it has been shown that manipulation of a tumor may result in a shower of tumor emboli.

Significance to Cancer Research:

Similar studies reported from other institutions were unsatisfactory in the following respects:

(1) The cytodiagnostic methods used were not shown to be quantitative (methods used here have been quantitated and shown to be highly efficient in recovering cancer cells from blood as noted in Part I, (E) of this report).

(2) No account was made for the possible "basic" variation in the numbers of cancer cells from one moment to another, independent of any of the events occurring in the operating room.

(3) The numbers of cases were too small.

Despite such inadequacies these reports have been the premise for recommended changes in operating procedure. By implication, a diagnostic curettage of endometrium has come to be considered perilous from the standpoint of causing hematogenous metastasis. Such an attitude, if not fully substantiated by further studies, could be damaging to patient welfare by leading physicians to abandon therapy or to needlessly embark upon major surgery for cancer without first obtaining essential diagnostic information not otherwise available. On the other hand, if the substance of such studies is supported we have need to consider whether any modification of present operating procedures might be indicated to help diminish hematogenous metastases, attributable to operating room procedures.

Proposed Course of Project:

Such studies will be carried out as often as the capacity of our laboratory personnel permits with the approval and cooperation of the operating surgeon.

Part I. (D) Evaluation of Methods of Study of Frequency and Significance of Tumor Cells in Blood. (Dr. West)

Objectives:

To determine what constitutes adequate sampling of blood to permit statistical analysis of factors associated with circulating cancer cells.

Methods Employed:

Methods are designed to show 1) whether time of day that a sample is collected is important; 2) the optimum number of samples of blood that must be studied in order to justifiably classify a patient as "positive" or "negative" for circulating tumor cells.

This study then will be mainly a statistical review of results previously obtained, and evaluated in the light of facts to be learned from (A) of this report.

Patient Material:

The patients of the National Cancer Institute on whom information regarding circulating tumor cells has been previously collected.

Major Findings:

In a review of circulating tumor cells in patients with choriocarcinoma it was noted that those patients classified as "negative" had an average of only three blood specimens examined, whereas those on whom at least one positive blood had been reported had had an average of seven specimens examined. A review has subsequently been made of circulating tumor cell studies on 377 National Cancer Institute patients who had from 1 to 37 peripheral blood specimens examined. The distribution of these patients according to the numbers of such examinations done, and the result as to whether or not at least one specimen was positive were as follows:

	No. of Patients	% of "positive" Patients
One Examination Only	183	10%
Two Examinations	80	27%
Three Examinations	35	31%
Four Examinations	14	22%
Five Examinations	15	53%
Six Examinations	5	80%
Seven Examinations	9	77%
More than Seven Examinations	36	72%
TOTAL	377	26.3%

An analysis of the case material by type and location of cancer shows that with epidermoid cancer of the head and neck and with gastrointestinal cancer, two groups which had an overall low incidence of circulating tumor cells in the peripheral blood, there was relative to the other types of cancer in this study, a disproportionate number of patients having only one blood specimen examined. This would tend to accentuate the proportion of "negatives" in those patients having only one specimen examined. However, this does not detract from the apparent indication that about six examinations of 20 cc blood samples are necessary in order to detect about 90% of all the patients who might be shown to have circulating tumor cells in the blood if an infinite number of samples were examined. This also shows that some certain number of examinations must be specified when any significance is to be attached to group of patients being classified as "positive" or "negative" for circulating tumor cells.

Significance to Cancer Research:

It has been common practice in reports relating to circulating tumor cells to classify patients as "positive" or "negative" without relationship to the numbers of the blood specimens examined or to the volumes of these specimens. Occasionally reports have even failed to distinguish between the general peripheral blood specimens and those collected from veins draining the cancer. These are obviously unsound practices which fact the present report substantiates. In spite of this, however, investigators have tried to relate prognosis to whether a patient was "positive" or "negative" for circulating tumor cells. The facts which seem to be supported by a retrospective study of data already collected should be further evaluated by a planned study. Probably such a study should be based upon a minimum six 20 cc samples of blood collected within a week's time, and any case not fulfilling this requirement should not be included since it is not reasonable to compare patients unless the data is collected under similar circumstances and with approximately equal sampling.

Proposed Course of Project:

When personnel capacities in the circulating tumor cell laboratories become available we plan to begin a new "population survey" type of study based on collection of at least six 20 cc samples of peripheral blood on each patient. The patients will be persons with known or suspected cancer. After passage of five or more years it may then become possible to relate the results of such studies to survival and to pattern and extent of metastasis.

Part I. (E) The Dextran Modification of Millipore Technique for Recovering Cancer Cells in Blood. (Dr. Romsdahl)

NCI-8-753(c)
Serial No.

Objectives:

Because various lysing agents, (Streptolysin-O, distilled water, Saponin or 2% acetic acid) usually either fail to lyse mouse erythrocytes, produce interferring protein sediments or cause excessive distortion of tumor cells, some other method of disposing of mouse erythrocytes was sought in order that mouse blood might be processed for recovery of circulating tumor cells. This was a necessary step in the conduct of studies of circulating tumor cells in mice. Rather than lysing the erythrocytes the present method relies upon rapid sedimentation of erythrocytes leaving essentially only leukocytes and tumor cells in suspension.

Methods Employed:

A counted number of cells derived from various mouse tumors (Sarcoma T-241, Sarcoma 49, Cloudman S-91 Melanoma) was suspended in 1 cc of mouse blood. To this tumor cell suspension was then added 1 cc 0.85% saline and 1 cc 6% Dextran (mollecular weight 188,000) these liquids mixed and allowed to settle for 40 minutes. The supernatant was carefully aspirated with a 10 cc syringe with an 18 gauge needle. The volume of the residual was restored to 1 cc by addition of 0.85% saline and then to this was added 1 cc each of 6% Dextran and 0.85% saline. The sedimentation and pipetting of the supernatant was repeated and the combined supernatants were centrifuged at 1500 RPM for 7 minutes, the sediment therefrom resuspended in 30 cc of 0.85% saline and centrifugation and resuspension repeated twice more. Finally 5 cc 10% formalin and 5 cc 0.85% saline were added to the plug obtained from centrifugation and the resultant suspension filtered at negative pressure 20 millimeters of Hg. through a Millipore filter with a pore size of 5 microns. The filters were fixed with Carnoy's solution, stained by Papanicolaou's technique mounted on glass slides and screened for circulating tumor cells, a quantitative count being rendered for each sample. The red cell mass obtained from the sedimentation in Dextran was resuspended in saline, lysed with 2 cc acetic acid and then centrifugation, resuspension fixation with formaldehyde and Millipore filtration and Papanicolaou's staining were carried out. No tumor cells were recovered from this portion of any of the samples.

Major Findings:

The Dextran sedimentation satisfactorily separated tumor cells and leukocytes from erythrocytes. Subsequent recovery and staining of tumor cells on Millipore filters yielded recovery of 92% ($\pm$ 8%) of the tumor cells and little or no distortion of the tumor cells occurred.

Significance to Cancer Research:

This study indicates that the Dextran Millipore filter method of

recovery of tumor cells from the blood of mice is a highly efficient method with recovery of almost 100% of suspended cells. This should then be a valuable research tool for quantitative study of circulating tumor cells in mice. If a comparable efficiency for the method should be demonstrated for human tumors suspended in human blood the method could be considered applicable for clinical investigation of circulating tumor cell problems.

Proposed Course of Project:

Similar studies will be carried out on a suspension of human tumor cells in human blood.

Part I. (F) A Clinical Study of Circulating Tumor Cells in Malignant Melanoma. (Dr. Romsdahl)

Objectives:

To study the relationship between progression of metastases in patients with malignant melanoma and the demonstration of circulating tumor cells in their blood.

Methods Employed:

Patients with histopathologically proven malignant melanomas were observed for progression of their disease as manifest by number and sizes of metastastic skin nodules and lymph node metastasis. These results were correlated with ability to demonstrate cancer cells in the blood using the technique previously described by Potter and Malmgren employing Streptolysin-O for lysis of red blood cells and granulocytes and Millipore filter for recovery of tumor cells, which were then stained by the Papanicolaou technique. Six patients with resectable melanomas were studied in regard to the presence of cells before, during and after extirpative surgery and the follow-up of these patients were noted for from 3 to 12 months.

Patient Material:

Forty-three patients of the National Cancer Institute with proven malignant melanomas were studied.

Major Findings:

There was generally a parallel in the increase in numbers and size of metastastic lesions with the frequency of recovery of tumor cells from the peripheral blood. 18% of 247 blood samples were positive for circulating tumor cells and of 36 patients with non-resectable melanomas, 25 (70%) had one or more specimens of blood positive for circulating tumor cells. Tumor cells in the blood were more frequent in the advanced cases, though occasionally positive blood for tumor cells was

obtained from patients with early lesions. In the six surgical cases tumor cells were found in the local blood at the time of surgery in only two, and these expired in 3 to 12 months respectively. One patient with positive postoperative blood specimens was alive and well without evidence of recurrence after 7 months.

Significance to Cancer Research:

Comparable numbers of specimens were not examined in each patient so that correlation of the patients progress to the presence or absence of demonstrable circulating tumor cells is not entirely valid. The parallel rise in frequency of demonstrable circulating tumor cells and an increase in numbers of metastatic lesions does not clearly indicate which is the cause and which the effect. For malignant melanoma in man, however, it seems that the demonstration of tumor cells in the blood is a sign of poor prognosis.

Proposed Course of Project:

We have no definite plans for continuing this study. If it is renewed, it will be done with more rigorous control of the study so that each case will be studied with equal intensity, and thus give comparable results.

Addendum:

Other activities of the circulating tumor cell laboratory have included:

1. Participation in a study of tumor cells in wound drainage involving the preparation and screening of about 650 Millipore filters.

2. Participation in a study on mice correlating the rate of disappearance from the blood of intravenously administrated Sarcoma T-241 cells with the rate of development of pulmonary metastases, involving the preparation and screening of about 200 Millipore filters.

3. Consultation services and assistance in projects involving cytological techniques which are being carried out by other members of the Surgery Branch of the National Cancer Institute.

Part I. (G) Prognostic Factors of Tumor Cells in Blood. (Drs. Potter and Retik)

Objectives:

To ascertain whether the presence of tumor cells in peripheral

and local blood of cancer patients have any prognostic significance.

Methods Employed:

Patients with various malignancies have had blood drawn from peripheral veins pre- and postoperatively and from local veins at the time of surgery. These bloods are processed and screened for the presence of tumor cells and patients have been followed to determine whether they have developed local tumor recurrence or distant metastasis.

Patient Material:

Patients with malignancies in the Cancer Institute and other institutes are studied.

Major Findings:

A review of the 140 patients is underway by Drs. Potter, Malmgren, Retik, and Smith. Definitive results are not available. The main problem in attempting to draw conclusions regarding the association of circulating tumor cells and prognosis is related to methods of sampling the blood. It is difficult to be sure when a patient is definitely positive or negative. Possibly all cases could be proven to have circulating tumor cells if the entire 4,000-5,000 cc of blood could be screened. When one is limited to small 20 cc samples, and the number of cells small, the statistical error can be great. Further development of basic design of the study, as described in Part D of this project, will probably be necessary if valid results are to be obtained.

From the study to date, however, it is possible to speculate that circulating tumor cells seem to be more frequently associated with the more advanced grade of malignant tumor, with those with less fibrosis, with more lymphocytic infiltration, and several other histologic parameters. However, it should be pointed out that this impression is purely a preliminary one, and not a proven fact.

From the clinical standpoint, we believe that there will be no correlation between finding of tumor cells in local blood at the time of surgery and prognosis of the patient. The significance of tumor cells in peripheral blood and outcome of disease remains even more obscure. Sampling errors in collecting the specimen, the stage of disease when obtained, the numbers of cells may all play a part. It is my personal opinion that this data will parallel that on wound washings and will probably show no correlation with prognosis or possibly only one associated with degree of contamination. Further work on this problem is under way.

Significance to Cancer Research:

This is another method of trying to determine the mechanisms of

metastasis and the relationship between tumor cells in the blood and the occurrence of distant metastases and local tumor recurrence.

Proposed Course of Project:

Follow-up data on 140 patients has been obtained and the study is still in progress.

NCI-8-753(c)
Serial No.

Project Title: Part II
Significance of Cancer Cells in Operative Wounds.

Principal Investigators: Dr. Marvin S. Arons

Other Investigators: Dr. Robert R. Smith and Mr. Max H. Myers

Cooperating Units: Biometry Branch, NCI

Project Description:

Objectives:

The objectives of this report were to show whether any relationship existed between cancer cells found in surgical wounds to survival, local recurrence, and distant metastases (spread below clavicle) as determined by life-table analysis.

Methods Employed:

This project has been underway for a number of years. Reference to previous reports will describe methods of study and preparation of washings. Wound washings are still being taken in all definitive cancer surgery cases. This report will deal mainly with the significance of cancer cells in wound washings. A total of 120 operative procedures were performed between September, 1953 and December, 1956 on a group of 111 patients, all of whom had primary invasive operable cancer without evidence of distant metastases (reported originally in 1958). This series of patients included 53 with head and neck cancer, 29 with female genital cancer, 11 with breast cancer, and the remaining 22 with abdominal cancer, soft part sarcoma, melanoma, or skin cancer. No patient was lost to follow-up which closed on August 1, 1960, giving a minimum follow-up of 44 months and a maximum of 83 months. Following surgical resection of the cancer, the operative site was sprayed with saline which was collected and prepared for cytologic examination. There were 26% positive washings, 60% negative, and 14% suspicious.

Major Findings:

Analysis of the data showed no correlation 1) between wound washing results and local recurrence, distant metastases or survival; 2) between positive lymph nodes and wound washing results; or 3) between positive lymph nodes and the incidence of local recurrence or distant metastases. However, there was a greater incidence of distant metastases in cases with local recurrence. In this series of cases wound washings proved to be of no prognostic significance.

A similar study was reported in Ann. Surg. regarding the role of operative wound seeding and recurrence in Head and Neck Surgery. As in the study described above, the finding of tumor emboli in wound washings could not be associated with the development of local recurrent cancer in the operative wound.

The incidence of positive wound washings remains about the same as that originally reported i.e., 25% in primary operable cancer cases.

Significance to Cancer Research:

The concept of local recurrence due to the seeding of cancer cells in operative wounds has long been recognized. As a practical index of seeding, wound washings were done to detect malignant cells. When such cells first were demonstrated, studies on adjunctive local wound chemotherapy were initiated. That study here showed no effect of .5% formaldehyde in preventing local recurrence or prolonging survival. The accumulation of this data emphasizes the need to explore new methods of therapy to control the implantation and growth of tumor emboli both locally in the wound and systemically at distant sites.

Proposed Course of Project:

It is intended to continue the study in order to show the relationship between the finding of tumor emboli in wound washings, wound drainage, circulating tumor cells and prognosis of the patient.

NCI-8-753(c)
Serial No.

Project Title: Part III
Tumor Cells in Postoperative Drainage.

Principal Investigators: Dr. Seymour C. Nash and Dr. Robert R. Smith

Other Investigators: Dr. Richard A. Malmgren and Mrs. Roxane Hume

Cooperating Units: Cytodiagnostic Service Path Anatomy Branch, NCI

Project Description:

Objectives:

Following the reports by Smith of tumor cells in wound washings it was felt that this finding would be of significance in predicting local wound recurrence of tumor. Follow-up data on those patients studied do not substantiate this however. This study was undertaken with two objectives. First, to demonstrate tumor cells in the post-operative drainage of patients undergoing radical cancer surgery and compare these results with the results of wound washings.

Secondly to determine if there is any relationship with time regarding the demonstration of such cells.

Methods Employed:

Postoperative drainage is collected via suction catheters from the wounds of all patients undergoing radical cancer surgery. The drainage is collected into a small trap bottle set in ice into which 4 cc. of Heparin and 2 cc. of Streptokinase-Streptodornase has been added. The mixture of Heparin and SKSD has proven to be effective in providing an easily filterable fluid. Collection is begun immediately after surgery and continued for several days. At various time intervals samples of fluid are removed and processed for tumor cells according to the Millipore filter method of Malmgren.

Major Findings:

Early results have demonstrated that tumor cells and clumps are indeed present in postoperative drainage. It has also been shown that tumor cells are present in the postoperative drainage of patients with negative wound washings at the time of surgery. In addition, tumor cells have been recovered as late as 43 hours after surgery.

Significance to Cancer Research:

These early results would indicate that this method used in addition to wound washings taken at surgery would provide a more effective guide in predicting future wound recurrence.

Proposed Course of Project:

This study is to be continued and expanded to include peripheral blood samples taken during times of collection and screened for circulating tumor cells. Since the method employed does not screen the entire postoperative drainage it is not quantitative. Using differential centrifugation it is anticipated that the entire specimen can be screened and quantitated.

Part B included Yes /XX/ No /__/

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Arons, Marvin S., Smith, Robert R., and Myers, Max H.: Significance of Cancer Cells in Operative Wounds. (Accepted for publication in Cancer).

Harris, Alvin H. and Smith, Robert R.: Operative Wound Seeding with Tumor Cells: Its Role in Recurrences of Head and Neck Cancer. Ann. Surg. 151:3, 330-334, 1960.

Potter, John F., Longenbaugh, George, Chu, Elizabeth, Dillon, John, Romsdahl, Marvin, and Malmgren, Richard A.: The Relationship of Tumor Type and Resectability to the Incidence of Cancer Cells in Blood. Surg. Gynec. & Obst. 110:6, 734-738, 1960.

Romsdahl, Marvin M., Potter, John F., Malmgren, Richard A., Chu, Elizabeth W., Brindley, Clyde O., and Smith, Robert R.: A Clinical Study of Circulating Tumor Cells in Malignant Melanoma. Surg. Gynec. & Obst. 111:6, 675-681, 1960.

Romsdahl, Marvin M., Chu, Elizabeth W., Hume, Roxane, and Smith, Robert R.: The Time of Metastases and Release of Circulating Tumor Cells as Determined in an Experimental System. (Accepted for publication in Cancer).

Romsdahl, Marvin M., Chu, Elizabeth W., Hume, Roxane and Smith, Robert R.: Method for the Cytological Detection of Circulating Tumor Cells in Mouse Blood (in vitro). (Accepted for publication in J.N.C.I.).

Serial No. NCI-8-754
1. Surgery Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Evaluation of the Nature, Cause and Means of Preventing Seeding of Operative Wounds Using Transplantable Animal Tumors.

Principal Investigator: Dr. Alfred S. Ketcham

Other Investigators: Dr. Seymour C. Nash

Cooperating Units: None

Man Years (calendar year 1960):
Total: 3
Professional: 1½
Other: 1½

Patient Days (calendar year 1960):

Project Description:

Objectives:

Part 1. A study of cytotoxic agents to determine their effect on altering the implantability of transplantable animal tumors. (Dr. Ketcham)

Part 2. The effect of 3:6 diamino acridine in preventing growth of transplantable tumor in operative wounds. (Dr. Nash)

Methods Employed:

Part 1. In previous studies reported under this project, it has been demonstrated that chemotherapy of cancer, as applied by the surgeon, required an agent to be effective on implants not yet growing in their metastatic site. This led to the development of artificially seeded operative wounds and the subcutaneous implantation of tumor inoculum by an air bubble technique. These techniques were applied in an *in vitro* group of studies in which the inoculum, after being removed from the donor animal, was pre-treated with the drug to be used before placing the inoculum into the test animal. This gives direct information regarding any oncolytic effect the drug under study may have when placed in direct contact with tumor cells.

In vivo studies more nearly approximate conditions seen in the treatment of human cancer by surgical means. In these instances the

tumor inoculum was placed in an operative wound, in an air bubble, intraperitoneally or intravenously. The drug to be studied was then applied at varying intervals of time. The effectiveness of the treatment can be measured by degree of inhibition of tumor growth. In all instances, toxicity studies were performed to determine the effect of the drug on normal tissues.

Previous reports have dealt with the effect of numerous agents on the animal tumor system, particularly Clorpactin XCB and Clorox. Preliminary evaluation of several other drugs was reported last year. This report will deal with an overall evaluation of some previously reported drugs as well as additional chemical compounds.

Tumor is prepared for implantation by taking one gram of viable tumor tissue from a donor mouse, passing it repeatedly through the Snell cytosieve to a 1:10 dilution, and then placing appropriate amounts of the inoculum either in the subcutaneous pouch raised with air on the back of the mouse or into an open axillary wound. Throughout the closed wound studies a .1 cc. of tumor suspension and ½ cc. of drug was used. In the open wound experiments .2 cc. of tumor suspension was used and 40 to 50 cc. of drug solution.

Within the closed wound studies, "pretreatment" evaluation of the drug was carried out. This entails placing the drug in the subcutaneous pocket at time intervals previous to placing the tumor into the pocket. This has also been carried out with open wounds in a similar fashion. The closed wound experiments were carried out using Carcinoma Krebs-2, Melanoma S-91 Cloudman, Sarcoma T-241 and Sarcoma DBA-49 each in their respective strain of mouse. Sarcoma T-241 was little affected by the drugs tried and so will not be included in this report. The open wounds had similar evaluation of these four animal tumor systems.

All animals were followed daily for the development of tumor in the wound and the day that tumor becomes palpable recorded and compared with the day that tumor becomes palpable in the control animals. They were then followed in an attempt to determine an alteration of their life span as related to treatment.

Part 2. Left axillary incisions simulating radical mastectomy wounds were made in DBA/P grey mice 7 to 14 weeks of age under sodium nembutal anesthesia. Wounds were then "seeded" using .05 cc. of a tumor suspension, of either S-91 Cloudman melanoma or mast cell tumor. Treatment consisted of 15 cc. of saline or 15 cc. of 3:6 diamino acridine 1:1,000 sprayed into the wounds over a 1 minute period 1-2½ hours after tumor inoculation. Other groups of animals were treated 3-3½ hours and 20 hours after inoculation. In addition, wounds were made bloody by cutting a small artery or vein and then treated with saline or 3:6 diamino acridine. Animals were allowed to succumb to tumor growth and death date and tumor appearance noted and compared.

Major Findings:

Part 1. Reference to Table I will strongly suggest that there is a consistent pre-treatment accentuation of tumor growth by a variety of drugs when they are placed in the wound before the inoculum. At a 5 minute pre-treatment time as well as a 30 minute pre-treatment time various concentrations of ThioTEPA, Clorpactin, Clorox, alcohol, hydrogen peroxide, Merthiolate and Dakin's Solution, allowed the tumor in the air bubble wound to become palpable before the tumor developed in the control animals. When the tumor was injected at 5 minutes or at 30 minute intervals before drug was brought into contact with the wound there was a rather consistent 1 to 8 day delay in the development of tumor. Concentrations of drug which were found by toxicity studies to be compatible with the animal when used in closed wound studies in no instance had a complete tumorcidal effect upon the tumor inoculum. Clorox 12% gave a depression whereby only 30% of the animals grew tumor at 5 minutes and 55% at 30 minutes following tumor inoculation.

The accompanying Tables II, III and IV, are the summarized results following tumor seeding of open axillary wound. While the volume of irrigation fluid remained constant at 40 to 50 cc. the manner of application was varied. In the first group, a syringe of 50 cc. size was filled with irrigating material and then the wound flooded gently for 15 to 20 seconds from this syringe. The low pressure spray in the second column of the accompanying Tables consists of spraying the wound again at a distance of about 6 inches with 5 pounds of pressure for 3 to 5 minutes. The third column, being the high pressure spray, was directed from a wall pressure valve estimated at 10 to 20 pounds of pressure for 1 to 2 minutes. Even though it was held 10 to 12 inches from the wound it often caused ballooning of the wound edges and subcutaneous dissection. The results of this study are in some aspects encouraging in that alcohol 50% resulted in only 5% and 8% respectively of the animals developing tumor with Krebs Carcinoma and Cloudman S-91 Melanoma. Other drugs such as .5% formaldehyde, 75% alcohol, 25% alcohol, 1.5% Dakin's Solution, and 12% Clorox gave less than 25% incidence of tumor growth. However, it is apparent that in no instance were we able to completely eliminate the growth of tumor in the wound. Possibly the more significant aspect of this study is seen in comparing the respective percentage of tumor takes between the animals as the pressure of applying the irrigant was increased. With the volume of fluid remaining constant but with the pressure of application of the irrigating fluid being increased from that of relatively no pressure from a syringe up to approximately 15 pounds of pressure, the incidence of takes increases in a step-wise fashion. As an example, alcohol 75% allowed a 14% takes with syringe spray, 43% with low pressure spray and 65% with high pressure spray, it may possibly be significant that saline has repeatedly decreased the percent of tumor takes to a range favorably comparable to most drugs tested. However, the incidence of tumor growth also increases with saline as the pressure

increases. The explanation for decrease and percentage of takes as the pressure increases can be best explained to one who has seen or carried out this technique. There is a driving force which may well imbed tumor cells into tissue planes while not allowing an adequate concentration of the drug to get into contact with the tumor cells. Why saline even with a high pressure spray results in a rather consistent similar percentage of takes can best be explained by imagining that some of the drugs may set up an irritative response in the tissue and allow a more nutritive bed for tumor cell propagation of those cells which were not killed by the original application of the tumorcidal agent. Throughout the open wound studies there is a consistent delay in the appearance of tumor as compared to the control but in all the studies the delay has not been felt to be significant nor has there been any significance in the survival rate of animals developing tumor after respective drug therapy to the wound.

Part 2. Mast Cell Tumor: Four percent of 49 animals treated with 3:6 diamino acridine 1-1½ hours after tumor inoculation developed tumor and died. The remaining animals are still alive without tumor up to 11 months post-therapy. All animals in the control group and saline treated group have developed tumor and died.

S-91 Melanoma: Initial experiments with S-91 Melanoma seeded wounds revealed that if the animals were sacrificed at 30 days none of the 3:6 diamino acridine treated group developed gross tumor. However, all animals had gross pigment in the wounds. Pathological examination of this pigment revealed it to be in macrophages. In all subsequent experiments the animals were allowed to succumb to tumor and tumor appearance date and death date noted.

Of 200 animals treated only 22 (11%) developed tumor as compared to 100% (123) controls and 99% (119/120) saline treated. In this group the latest appearance of tumor was 61 days after treatment. In another experiment 10 treated animals were sacrificed at 70 days, and pigment found to present in the wounds. One half of this pigment was submitted for microscopic study and ½ retransplanted into 10 recipient animals. The pigment was seen microscopically to be intracellular in macrophages and extracellular. The retransplanted pigment did not grow. This again suggests that the pigment was not contained in dormant tumor cells.

If wounds were treated 3-3½ hours post-tumor inoculation the percent of tumor take rose to 50%, treatment 20 hours post-inoculation produced an incidence of 84% take. When wounds were made bloody prior to treatment, 52% take occurred.

Significance to Cancer Research:

Part 1. As further laboratory and clinical experience with

local chemotherapy of seeded operative wounds is obtained, it becomes apparent that more is involved in implantation and growth of tumor implants than the simple presence of the cells. The ability of implants to grow can be influenced in both a positive and a negative way. Simple application of tumorcidal agents is apparently not sufficient to control local recurrence of cancer unless 3:6 diamino acridine turns out to be an exception.

Proposed Course of Project:

The overall study has been expanded with the two investigators working jointly together to include the relationship of time of treatment after inoculation with special reference to saline spray and to the percentage of takes and the relationship of known number of tumor cell inoculations to concentrations in amount of drugs or saline used.

Part B included Yes /XX/ No /___/

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Ketcham, Alfred S.: The Use of a Hypochlorous Acid Derivative in the Control of Cancer: An Animal Investigation of Seeded Wounds. Cancer. 13:1, 20-24, 1960.

Honors and Awards relating to this project: None

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Serial No.

TABLE 1

IN VIVO TREATMENT OF CLOSED SEEDED ANIMAL WOUNDS - CARCINOMA KREBS-2, MELANOMA S-91 CLOUDMAN, SARCOMA DBA-49
% OF ANIMALS THAT DEVELOPED TUMOR

Drug	Drug Injected before Tumor		Avg.	Tumor Injected before Drug		Avg.	No. of
	5 Minutes	30 Minutes	Day*	5 Minutes	30 Minutes	Day**	Wounds
Saline (Isotonic)	99%	100%	-1	98%	100%	+1	120
ThioTEPA .05%	100%	92%	-2	94%	92%	+6	119
ThioTEPA 0.1%	92%	90%	-2	80%	74%	+7	65
Clorpactin 2%	90%	85%	-4	80%	86%	+2	101
Clorpactin 1%	95%	90%	-3	82%	90%	+2	114
Clorox 12%	70%	82%	-3	30%	55%	+2	92
Clorox 6%	74%	70%	-4	55%	68%	+4	115
Clorox 3%	90%	88%	-2	72%	81%	+3	120
Clorox 1.5%	94%	92%	-1	90%	92%	+2	120
Alcohol 25%	76%	80%	-3	60%	65%	+3	118
H_2O_2 1%	75%	80%	-4	69%	70%	+2	114
Merthiolate .2%	85%	90%	-5	72%	68%	+3	80
Dakin's Sol. .5%	72%	68%	-2	50%	60%	+9	99
Dakin's Sol. .25%	71%	75%	-3	68%	65%	+8	106

* Day tumor appeared in treated animals before it appeared in controls
**Day tumor appeared in treated animals after it appeared in controls

98% of 350 control animals developed tumor

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TABLE 2
IRRIGATION OF TUMOR SEEDED AXILLARY WOUNDS

Drug	% OF ANIMALS WITH TUMOR GROWTH						No. of Wounds	Day (Avg) Delay in Tumor Appearances
	Carcinoma Kreb-2			Cloudman S-91 Melanoma				
	Syringe	Low Pressure	High Pressure	Syringe	Low Pressure	High Pressure		
Saline (Isotonic)	20	55	58	20	55	59	354	5
Dist. H_2O	21	30	37	29	68	60	352	5
Saline (100 cc.)	18	30	63	8	29	41	230	4
ThioTEPA	36	85	88	10	11	54	86	6
ThioTEPA + Saline	48	60	79	19	45	56	90	9
ThioTEPA + H_2O	40	84	70	26	41	50	92	7
Phenol 0.1%	62	94	84	54	77	84	108	6
Formaldehyde 0.5%	21	50	51	14	41	50	104	15
Alcohol 75%	14	43	65	10	30	55	96	14
Alcohol + Saline	24	48	60	11	34	60	98	11
Alcohol 50%	5	40	48	8	35	45	102	10
Alcohol + Saline	8	50	40	16	36	48	114	9
Alcohol 25%	19	45	51	12	30	65	116	7
Alcohol + Saline	21	60	56	16	41	45	112	10

95-100% of 350 Control Animals Developed Tumor

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TABLE 3
IRRIGATION OF TUMOR SEEDED AXILLARY WOUNDS

% OF ANIMALS WITH TUMOR GROWTH

Drug	Carcinoma Kreb-2			Cloudman S-91 Melanoma			No. of Wounds	Day (Avg) Delay in Tumor Appearances
	Syringe	Low Pressure	High Pressure	Syringe	Low Pressure	High Pressure		
Merthiolate .22%	41	71	77	29	52	72	102	10
Clorox 12%	24	55	69	18	45	70	92	7
Clorox 6%	30	50	50	22	44	65	100	7
Clorox 3%	26	48	58	24	51	72	110	6
Clorox + Saline	28	42	71	28	49	68	108	7
Clorpactin 2%	27	49	60	30	58	62	81	7
Clorpactin + Saline	38	50	52	22	59	68	74	9
Clorpactin 1%	32	54	67	51	62	78	90	8
Clorpactin + Saline	32	58	62	30	70	81	88	6
Clorpactin 0.5%	31	60	68	12	68	71	100	10
Clorpactin .25%	30	65	78	30	72	88	108	7
Clorpactin + Saline	15	60	72	28	51	69	112	7
Dakin's Sol. 1.5%	18	33	45	19	40	54	110	11

95-100% of 350 Control Animals Developed Tumor

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TABLE 4

IRRIGATION OF TUMOR SEEDED AXILLARY WOUNDS

% OF ANIMALS WITH TUMOR GROWTH

Drug	Sarcoma T241m		Sarcoma DBA-49		Avg. Day Delay in Tumor Appearances	No. of Wounds
	Syringe	Low Pressure	Syringe	Low Pressure		
Control	100	100				160
Saline	28	45	98	98	3	78
Saline (100 cc.)	18	38	23	60	4	70
ThioTEPA 0.1%	54	76	16	49	6	52
Phenol 0.1%	42	84	48	69	3½	82
Formaldehyde .5%	38	58	51	89	6	64
Alcohol 75%	22	48	31	48	7	68
Alcohol 50%	20	46	27	49	6	72
Alcohol 25%	22	54	21	54	8	78
Clorox 6%	28	39	19	60	10	70
Clorox 3%	31	42	22	56	10	76
Clorpactin 2%	26	50	28	50	8½	48
Dakin's Sol. .5%	20	44	19	39	9½	76

Serial No. NCI-8-755(c)
1. Surgery Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Use of a Chemically-Defined Water Soluble Diet in Cancer Patients.

Principal Investigator: Dr. Robert B. Couch

Other Investigators: Dr. Leon Rosenberg, Dr. Milton Winitz, Dr. Sanford Birnbaum, Mr. M. Clyde Otey

Cooperating Units: Laboratory of Biochemistry and Metabolism Service, NCI

Man Years (calendar year 1960):
Total: ½
Professional: ½
Other: 0

Patient Days (calendar year 1960):

Project Description:

Objectives:

The purification and synthesis of amino acids, carbohydrates, fats, and vitamins has allowed the nutritional investigator considerable freedom of manipulation of these essential nutrients and has aided in maintaining constancy of diets. Liquid diets, by providing uniform intake, enhance the quantitative validity of nutritional investigation. Greenstein and associates have added still another advantage to quantitative studies by designing an entirely water soluble diet. This insures equal exposure of all nutrients to intestinal mucosa as well as making diet analysis more accurate. These investigators have utilized the advantages of a chemically defined water soluble diet for quantitative nutritional studies in rats. Their investigation has been focused primarily on the amino acid content, and comparisons of ratios, quantities, and composition of this fraction have provided many illuminating features of amino acid metabolism. This project was designed in order to expand to humans these studies as well as numerous others. This report constitutes selection and study of a form of this diet suitable for clinical investigation.

I. Design of Diet:

All vitamins and minerals used in this diet were felt to be essential for man. The mineral salts used were selected for solubility reasons. All quantities used were those recommended by the food and nutrition board of the National Research Council. Where no recommendation was available the literature was consulted and a reasonable quantity arrived at. Four mixtures of amino acids were used (Table I). Mixtures 1 and 2 were used in the preliminary studies and mixtures 3 and 4 in the metabolic balance studies. Mixture 1 includes all ten amino acids essential for the rat. Arginine was added because of the reported value in preventing ammonia toxicity. Histidine was included because only short term human studies have been performed without this amino acid. In preliminary studies DL Alanine was used as a source of nonessential nitrogen to make a total quantity of 75 grams of amino acids with a calculated nitrogen value of 11.2 grams per day. Mixture 2 was used in one patient. Mixture 3 was the basic mixture for balance study and consisted of the 18 amino acids present in casein. The ratio of essential amino acids used were those resulting from determination of the minimum requirement of each essential amino acid for 120 gram rats feeding solely on this type diet. The concentration of essential amino acids, 37% of the total quantity, was shown to produce optimal growth in the rat. The total quantity of each essential amino acid given per day was greater than the minimum intake determined by Rose. Ediol* was used as a source of fat in three instances to provide 1/3 of calories. This was later changed to ethyl linoleate, thus providing all nutrients in chemically defined form. The total lipid was the only nutrient in this diet present in less than optimal quantities. Dextrose, sucrose, and maltose were all used as a carbohydrate source. The only difference noted was the difference in relative sweetness when given orally. Dextrose was selected as the most desirable source of carbohydrate. The total daily diet aliquot provided 40 calories per kilogram weight except as noted.

II. Preparation and Administration of Diet:

All amino acids and monocalcium fructose 1,6-diphosphate were purified by dissolving in boiling water or hot alcohol, filtering through activated charcoal, and reprecipitating with 95% alcohol or vacuum distillation. The precipitate was filtered and washed with alcohol and ether. Sugar, vitamins, and minerals used were all of the highest purity obtainable. Ediol was obtained from Schenlabs Pharmaceuticals, Inc. Ethyl linoleate was obtained from the Hormel Research Institute and was 99% pure.

* Commercially available coconut oil mixture.

The diet was prepared in two ways, For the preliminary studies the chemicals were weighed and bottled in dry form in daily aliquots. These were put into solution in a Waring blendor and filtered through Whatman #4 filter paper. The sugar was placed in solution separately and the two solutions mixed. For the patients receiving Ediol, daily aliquots were bottled and the fat and fat soluble vitamins added on the day of use. In the remaining preliminary studies the ethyl linoleate and fat soluble vitamins were mixed thoroughly with polysorbate 80, then mixed with the diet to produce an emulsion. Daily aliquots were measured volumetrically and refrigerated until used.

For the balance studies the less soluble amino acids were placed in solution by shaking a 5 gallon jug containing an aliquot of distilled water. The more soluble amino acids, minerals, and vitamins were then placed into solution in a similar manner. Dextrose, dissolved separately, was added to the solution and shaking was continued until the solution was water clear. The diet was filtered through #4 filter paper, and ethyl linoleate and fat soluble vitamins added in the same manner as noted above. Daily aliquots were removed and frozen until 12 hours prior to use. They were then thawed, 200 cc. of 2% carboxmethylcellulose and flavoring (when used) were added, and the aliquot diluted to a predetermined 20 to 25% diet solution. Five separate feedings were measured out and refrigerated until given.

The diet was given in 12 separate courses to 7 patients. Five patients received the diet via indwelling esophagostomy tube and two patients received the diet orally. When given orally, flavoring was given to improve taste. Patients in the preliminary study received the diet in 3 to 8 separate feedings, and the two patients during metabolic balance study received the diet in 5 separate feedings per day. The feeding was taken over a 10 to 30 minute period. Distilled water was provided ad lib except during metabolic balance study where a regimented intake was used.

III. Design of Study:

1. Preliminary studies: Six patients received the diet for 20 days with 14 days of control before and after each experimental period. Two patients received the diet for 7 days only after the observation was made that all problems arose within this time limit. Weight was taken accurately every other day. A record was kept of each stool with its description and stool weights and volumes were measured on one male patient during the experimental period. Intake and output records were kept on five patients. Urine and blood studies were performed once weekly and included routine urinalysis,

complete peripheral blood counts, and a broad coverage of blood chemistries. Control diet was regular hospital or Waring blendor diet. Two patients received 30 calories per kilo during the control periods and one patient on a weight reducing regime received 1200 calories per day.

2. Variations in calories and amino isomers: Patient E. C. was selected for this study. Control diet was Waring blendor. Each study consisted of two 6 day periods with two or three control periods before and after each. Three forms of the diet were compared in this setting. The diet as listed with amino acid mixture number 3 at 40 calories per kilo, the same diet with added dextrose to make 50 calories per kilo, and the diet as listed with amino acid mixture number 4 were used in separate 12 day study periods. All diets, experimental and control, were isonitrogenous. All were isocaloric except as noted. Standard metabolic balance techniques were used. Diet and pooled urines and stools were analyzed for total nitrogen, uric acid, K^+, Na^+, Cl^-, Ca^{++} and p^{+++}. Bloods were drawn and analyzed one per period for TBS, BUN, creatinine, uric acid, ammonia, TSP A/G, Na^+, K^+, Cl^-, CO_2, Hct, Hgb, and WBC. Serum lipids consisting of cholesterol (total and esters), phospholipids, triglycerides, and unsaturated fatty acids were performed once per period. Theoretical and actual balance were calculated and results of each period compared.

3. Depletion-Repletion: Patient J. G. was selected for this study. Following a 12 day control period he was placed on a chemically defined water soluble nitrogen free diet for 12 days of nitrogen depletion. This was followed by 12 days of nitrogen repletion utilizing the same diet with amino acid mixture number 4. All diets were isocaloric throughout (40 calories/kilo), and the repletion diet and control diets were also isonitrogenous. Standard metabolic balance techniques were used and the same data obtained as above.

Patient Material:

These studies were performed on seven patients of the surgical service. An effort was made to select them at a time when no extraneous influences would occur during the study period. Other than this, the only criterion for selection was that they be stable in their disease process, cooperative, and have an intact GI tract and normal liver and renal function studies. A brief case history of the two patients on whom metabolic studies were performed will be described.

Patient E. C. (45 CM): Admitted to NIH in 1954. This patient gave a history of primary lip carcinoma in 1945 with wedge resection followed by irradiation of a recurrence. There was no further treatment until 1954 when he presented himself at the NIH with extensive local involvement and bilateral cervical node metastases. A radical local excision with bilateral neck dissection was done. Since that time he has had numerous excisions for local recurrences. His weight has been stable. At the time of this study he had extensive local recurrence of his cancer associated with a great deal of pain. There was no evidence of distant metastases.

Patient J. G. (69 WM): This man presented himself at the NIH in 1957 with 4 x 4 cm. fungating epidermoid carcinoma of the hard palate. A radical local excision was done. Since then his weight has been stable and no evidence of recurrence has been noted.

Major Findings:

I. Preliminary Studies:

1. Clinical findings: All three patients receiving Ediol as a source of fat complained of occasional abdominal cramps. This has not been present since it was eliminated. Excess flatus and eructation were frequently reported, though clinically insignificant symptoms. Preliminary observations limited the difficulty to two symptoms, nausea and diarrhea. To elucidate their origin a series of elimination diets were set up designed to point to the cause of these symptoms. The sugar used in each diet bore no relationship to symptoms except that with sucrose the diet was reported as "nauseously sweet." Schedule of administration, Ediol, polysorbate 80, and D amino acids bore no relationship to nausea or diarrhea. The paramount factor as regards nausea proved to be diet concentration. Diet solutions of 35-65% produced nausea in four of five patients. Diet solutions of 20-25% failed to produce any nausea in three patients. When nausea did occur, an apparent physiological adjustment took place and the symptom would leave after 5 to 7 days.

Diarrhea could be more appropriately labeled "frequent call to stool" as measured volumes were always less than 200 cc. and weights less than 200 grams per 24 hours. However, it was an annoying symptom. On many days the patients would have 5 to 7 stools and in one patient as many as 12 per day. All stools were mucoid and small, and this symptom remained present throughout the experimental period. No specific cause has been discovered; however, when carboxymethylcellulose was added to all the special diet of one patient, diarrhea did not occur.

2. Weights: Two patients lost weight throughout the course of the study, one on 1200 calories and one in a prolonged postoperative state. These weight records are not included here. The two patients receiving the diet for seven days only did not lose weight. The four cases presented here had no clinical or laboratory reason to lose or gain weight apart from the use of this diet. Patient E. C. had a steady gain in weight throughout the study with a total of one kilogram gain for the 48 day period. Patient M. C. had no change throughout. Patient M. F. lost 1.0 kilograms in the control period in association with a local surgical procedure. Following stabilization, the special diet was begun and she had no further weight loss. Patient J. G. lost 1.0 kilogram over the initial seven days of the experimental period. This was associated with clinically significant nausea. The regime was not changed, nausea disappeared after seven days, and he had no further difficulty. This was followed by regain of the weight loss.

3. Others: Intake and output records revealed no abnormal water balance. All of urine and blood analyses remained essentially unchanged throughout the 48 day period with the exception of blood urea nitrogen. This chemistry decreased during the experimental period in 5 of the 6 patients. Decreased urea synthesis has been suggested by the decreased urea excretion reported by Birnbaum with the use of a DL amino acid diet.

II. Variation in Calories and Amino Acid Isomers:

Patient E. C. was the subject. There was no significant change in weight or abnormal water balance. The patient experienced nausea with 25% diet concentrations requiring complete cessation of intake for 4/5 of one day. On decreasing concentration to 17-20%, no further nausea occurred. Whereas normally the patient had 1 to 2 stools per day, while receiving the experimental diet he had 2 to 3. Otherwise no clinical abnormalities were noted.

Nitrogen Balance: Period 1 followed reduction from a voluntary intake of 5,000 calories and 125 grams of protein per day and was felt to represent readjustment. The diet used in periods 4 and 5 was isonitrogenous and isocaloric with control diet. It is apparent that nitrogen retention was less on an L amino acid diet than on a natural food diet. The average value of nitrogen retained per day was 0.6 grams less for the L amino acid diet than for control diet. The experiment diet used in periods 9 and 10 was an isonitrogenous L amino acid diet providing 10 calories per kilogram weight more than control diet. The use of this diet resulted in nitrogen retention which was not significantly different than the control diet. When the isocaloric L amino acid diet containing the less

costly DL form was employed there was an initial negative nitrogen balance averaging 1.9 grams per day. However in the following 6 day period nitrogen was retained at the rate of 2 grams per day. The resultant nitrogen retention during periods 13 and 14 make this diet clearly inferior to all others. It is felt that the positive nitrogen balance on followup control was a manifestation of repletion.

Analysis of pooled urines during various periods of synthetic diet administration revealed that alpha amino acid nitrogen output during periods 4-5 and 9-10 remained within the normal range. However during the DL diet of periods 13 and 14 there was a tenfold increase in excretion of amino acids with values of 1000 to 1100 mgms. being obtained during both periods. There was no fall in output during periods 14 to correspond with return to positive nitrogen balance. An initial decrease in weight of the subject occurred with institution of each synthetic diet. The weight was recovered without change in diet in the first two instances but not with the diet containing DL amino acids. These figures appear to underline clearly the inferior nature of diets containing DL amino acids.

Other Balance Data: Calorie balance data revealed a relatively positive energy balance during periods 4-5 and 9-10 as compared to control diet. The explanation for this is uncertain but during administration of the DL diet caloric balance was relatively negative.

No conclusive changes were noted in mineral balance or uric acid changes. A uricosuric effect of amino acids was suggested from these data but not confirmed by the subsequent study.

Fat studies revealed a consistent increase in Triglyceride levels with each experimental diet but otherwise no consistent changes.

One can conclude from this study that natural foods have a greater capacity for producing nitrogen retention in man than this chemically defined water soluble diet, that with the addition of more carbohydrate nitrogen retention produced by this chemically defined diet can be made equal to natural food, and that the addition of D amino acids to this diet results in an initial negative nitrogen balance that offsets the advantage of decreased cost.

III. Depletion-Repletion:

Patient J. G. was the subject. Apart from urgency to stool and increase in frequency of 1-2 stools per day, the only symptom was that of mild lassitude during depletion which the patient was not aware of until he noted the difference during the repletion phase.

Nitrogen Balance: During depletion the patient was in negative nitrogen balance by 3.61 grams per day. During repletion positive nitrogen balance was afforded at a rate of 2.91 grams per day. On followup control repletion continued but at a slower rate. Although nitrogen balance was stable it should be noted that the subject began to gain weight after being placed again on natural foods.

The alpha amino acid nitrogen output fell by approximately 30% during depletion, but total output in all periods remained within normal limits.

Others: Caloric balance was relatively more negative than control diet, thus paralleling use of the DL diet in the previous study. No explanation is apparent. No pertinent changes in mineral balance was noted except an initial sodium retention with experimental diet reflected in a weight gain. Serum uric acid decreased with intake of experimental diet as in the previous study but this was not reflected in an increased output of uric acid. A similar increase in triglyceride levels occurred as in the previous study.

One can conclude from this study that the diet will satisfactorily replete a nitrogen subject.

Significance to Cancer Research:

These studies provide baseline information and data on an experimental tool that can be used to ascertain the nature of amino acid and nitrogen metabolism in cancer patients. Ascertaining these abnormalities not only will further our understanding of cancer, but may lead the way to potential avenues of therapy.

Proposed Course of Project:

Publication of the data presented is planned. Further studies in the metabolism of cancer will await return of the senior author from temporary leave of absence.

Part B included: Yes /__/ No /XXX/

TABLE I

Diets Utilized in This Study

Diet 1

Vitamins:	Thiamine HCl	1.2 mgm
	Riboflavin	1.8 mgm
	Pyridoxine HCl	2 mgm
	Niacin	12 mgm
	Inositol	1 mgm
	Choline HCl	150 mgm
	Ca. Pantothenate	10 mgm
	Biotin	1 mgm
	Folic Acid	2 mgm
	Ascorbic Acid	75 mgm
	Vitamin B_{12}	2 mgm
	PABA	500 mgm
	Vitamin A	4000 i.u.
	Vitamin D	800 i.u.
	Tocophenol	30 mgm
	Vitamin K	2 mgm
Minerals:	Potassium Citrate	12 gms
	Magnesium Citrate	0.25 gms
	*Potassium Hydroxide	2.4 gms
	*Magnesium Oxide	0.45 gms
	*Glucuronolactone	10.8 gms
	Potassium Iodide	0.3 mgm
	Manganese Acetate	22 mgm
	Zinc Benzoate	2.5 mgm
	Cupric Acetate	3 mgm
	Cobaltous Acetate	2 mgm
	Ammonium Molybdate	0.5 mgm
	Ferrous Ammonium Sulfate	84 mgm
	*Ferrous Gluconate	0.16 mgm
	Sodium Chloride	5 gms
	Monocalcium Fructose 1,6-Diphosphate	50 gms
Carbohydrates:	Dextrose	
	*Sucrose	remaining
	*Maltose	calories
Fats:	Ediol	1/3 calories
	*Ethyl Linoleate	2 gms

Amino Acids:	DL Tryptophane	1 gm
	DL Phenylalanine	4.4 gms
	DL Threonine	2 gms
	DL Valine	3.2 gms
	DL Methionine	4.4 gms
	L Isoleucine (D Alloisoleucine(	2.8 gms
	L Lysine	1.6 gms
	L Leucine	2.2 gms
	L Arginine HCl	2 gms
	L Histidine HCl	1.5 gms
	DL Alamine	50 gms

Diet 2

Vitamins: Same as Diet 1

Minerals: Same as Diet 1

Carbohydrate: Maltose

Fat: Ethyl Linoleate

Amino Acids:	L Tryptophane	0.5 gm
	L Phenylalanine	2.2 gms
	L Threonine	1.0 gm
	L Valine	1.6 gms
	L Methionine	2.2 gms
	L Isoleucine	1.4 gms
	L Lysine HCl	1.6 gms
	L Leucine	2.2 gms
	L Arginine HCl	2.0 gms
	L Histidine HCl	1.5 gms
	L Alanine	50 gms

Diet 3

Vitamins: Same as Diet 1

Minerals: Same as Diet 1

Carbohydrate: Dextrose

Fat: Ethyl Linoleate

		% Total Nitrogen
Amino Acids:	L Lysine HCl	19.16
	L Histidine HCl	27.08
	L Arginine HCl	32.16
	L Tryptophane	13.72
	L Phenylalanine	8.48
	L Leucine	10.68
	L Isoleucine	10.68
	L Threonine	11.76
	L Methionine	9.36
	L Valine	11.96
	L Alanine	15.72
	Glycine	18.66
	L Aspartic Acid	10.52
	L Proline	12.17
	L Monosodium (	8.28
	L Glutamate (	
	L Glutamine	12.33
	L Tyrosin Ethyl Ester HCl	5.70
	L Serine	13.33
	L Cysteine Ethyl Ester HCl	7.54

Diet 4

Vitamins: Same as Diet 1

Minerals: Same as Diet 1

Carbohydrate: Dextrose

Fat: Ethyl Linoleate

Amino Acids: Same amino acids as Diet 3. All isomers were racemic DL form in twice the value given for essential amino acids and the same value for nonessential amino acids with the exception of those listed. These amino acids were L form and were used in the same quantities as in Diet 3.

L Lysine HCl
L Histidine HCl
L Arginine HCl
L Aspartic Acid
L Proline
Monosodium L Glutamate
L Tyrosin Ethyl Ester HCl
L Cysteine Ethyl Ester HCl

TABLE II

Nitrogen Balance Data

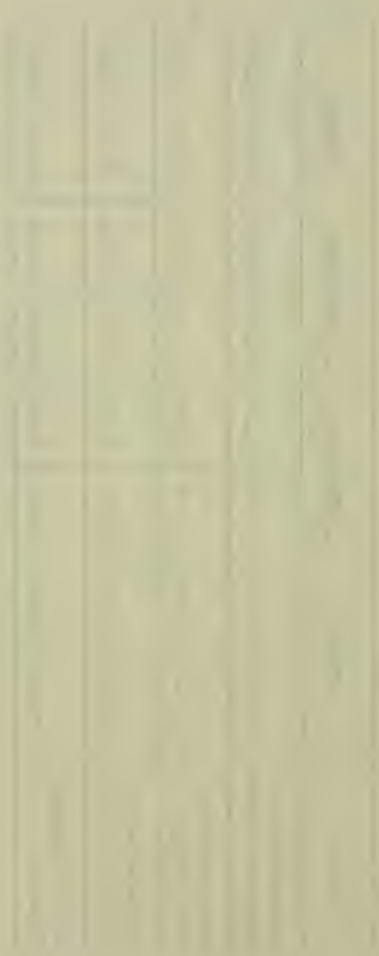
A. Variations in Diet

Period	Control		L aa at 40 cal/kg		Control			L aa at 50 cal/kg		Control		DL aa at 40 cal/kg		Control
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Average Daily Nitrogen Intake	10.32	10.32	10.32	10.32	10.86	10.86	10.86	8.88	10.25	11.03	11.03	11.10	11.10	10.20
Average Daily Stool Output	1.09	1.22	.33	.58	1.34	1.06	.85	.84	.60	.74	.72		1.27	.17
Average Daily Urinary Output	7.19	7.62	9.18	8.21	6.82	6.98	8.72	6.62	6.80	7.36	7.85	11.60	7.78	6.64
Daily Nitrogen Balance	+2.04	+1.48	* +.80	+1.52	+2.70	+2.82	+1.29	* +1.48	+2.85	+2.94	+2.46		+2.04	+3.38

TABLE II (Continued)

B. Depletion-Repletion

Period	Control		Depletion		Repletion		Control	
	1	2	3	4	5	6	7	8
Average Daily Nitrogen Intake	11.13	11.13	.26	.25	10.93	10.93	11.04	11.04
Average Daily Stool Output	1.23	1.98	.48	.66	.59	.98	1.31	1.22
Average Daily Urinary Output	8.50	7.31	3.85	2.72	7.42	7.06	7.16	7.04
Daily Nitrogen Balance	+1.40	+1.83	-4.07	-3.14	+2.92	+2.89	+2.56	+2.78



Serial No. NCI-9-756(c)
1. Surgery Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Viruses Associated with Human Tumors.

Principal Investigators: Dr. Robert A. Manaker, Laboratory of Biology, NCI, Dr. Edward McLaughlin, and Dr. Robert B. Couch

Other Investigators:

Cooperating Units: Laboratory of Biology, NCI

Man Years (calendar year 1960):
Total: ½
Professional: ½
Other: 0

Patient Days (calendar year 1960):

Project Description:

Objectives:

Studies have demonstrated that viruses may be associated with animal tumors in any of several ways. The virus may be only a passenger serving no beneficial or detrimental relationship with the tumor. It may be a latent infection of varying duration, activity when it occurs being manifested by necrosis of tumor or stimulation of growth. Secondarily infected tumors by artifical or natural means may result in tumor necrosis and finally, viruses infecting normal tissue with or without intervening latent period may induce tumor formation in the tissue. Many of these relationships have been demonstrated in virus-animal tumor systems, while some have also been demonstrated in virus-human tumor systems. In order to study these virus-human tumor relationships cooperative studies were instituted between the Surgery Branch of the NCI and the Laboratory of Biology, NCI. It has as its objective the isolation of viruses from human tumors and the characterization of the relationship that existed. The effort of the Surgery Branch has been directed by the premise that the virus might be unmasked and/or produced in sufficient quantity for study by maintaining the parent tumor in a host free system. This system has consisted of tissue cultures of the tumors in heterologous or artifical media.

Materials:

1. Hanks Basal Salt Solution

2. NCTC 109 Medium
3. Eagle's Medium
4. Pooled Human Serum (contract)
5. Horse Serum
6. Penicillin and Streptomycin
7. T-15 Culture Flasks

Methods Employed:

1. The tumor immediately after excision is cut by a pathologist under sterile conditions and a portion placed in chilled basal salt solution and delivered to the Laboratory of Biology, NCI (Dr. Manaker).

2. A wedge of tumor approximately 1 x 1 x ½ centimeters is minced and washed three times in chilled Hanks Basal Solution, with centrifuging and discarding of the supernant fluid between washes.

3. After the third washing, the tumor tissue is transferred into T-15 flasks where it is evenly distributed. Three milliliters of the specific nutrient material is placed in each flask.

4. The T-flasks are incubated at 37° C., observations being made three times weekly and fluids repleted, saved, or changed depending upon what occurs. At the end of four weeks, if no growth has occurred, the T-flasks are terminated. If any spontaneous degeneration of tumor or cytopathogenic effect is seen, a portion of the tissue fluid is saved, (frozen) to be used for inoculating on indicator lines.

Patient Material:

Twenty-seven primary tumors have been evaluated in the past 12 months. All were removed at surgery from patients of the Cancer Institute. Only primary tumors are used.

Major Findings:

Growth in T-flask was obtained in the twenty-seven specimens in one or more T-flasks prepared for each specimen. However, none of the twenty-seven specimens were carried longer than 32 days by the methods presently employed. Contamination by bacteria has occurred occasionally while contamination by fungus, although occurring sporadically, continues to present a major problem.

Significance to Cancer Research:

With the current popularity of the theory that viruses are the etiological agents of human cancer, study of the virus-tumor relationship in humans is fundamental to the understanding of cancer.

NCI-9-756(c)
Serial No.

Proposed Course of Project:

It is planned to continue to obtain specimens of primary cancer for further evaluation of the possible virus-human tumor relationship.

Part B included Yes / / No / X /

Serial No. NCI-10-759(c)
1. Surgery Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calenday Year 1960

Project Title: Experimental Studies of Mechanism of Metastases.

Principal Investigators: Dr. Alfred Ketcham, Dr. Robert R. Smith.

Other Investigators: Dr. Robert C. Hoye, Dr. D. L. Kinsey, Dr. Marvin Romsdahl, Dr. George F. Miller, H. Wexler, R. Hume.

Cooperating Units: None

Man Years (calendar year 1960):
Total: 3
Professional: 1½
Other: 1½

Patient Days (calendar year 1960)

Project Description:

Objectives:

This project is made up of a number of studies by a group of investigators in an attempt to show the effect of both intrinsic as well as extrinsic factors on the incidence of artificial and spontaneous lung metastases in experimental animals.

Part I. (Dr. Ketcham)

a. The effect of complete primary tumor removal.
b. The effect of incomplete tumor removal and trauma.
c. The effect of the age of the host.
d. The effect of nutritional changes.
e. The effect of denervation of an extremity (Miller).
f. Effect that additional tumor systems will have.

Part II. (Dr. Hoye)

The effectiveness of small amounts of irradiation in decreasing the number of metastases.

Part III. (Dr. Kinsey)

The demonstration of Preferential Metastases.

Part IV. (Dr. Romsdahl)

The Relationship of Circulating Tumor Cells and Metastases.

Part V. (Dr. Arons)

The Effect of a Foreign Body on Metastases and Tumor Spread on the Foreign Body.

Part I.

Methods Employed:

(a) Effect of primary tumor removal; In the past year efforts have been continued to confirm the previously reported findings resulting from the standardization of several tumor host systems. Melanoma S-91 Cloudman, DBA-49 Sarcoma, T-241 Sarcoma, Amelanotic S-91 Melanoma, and Osteogenic Sarcoma 112 have been under continued experimental observation.

These tumors are all carried in our laboratory. Inoculums from these tumors for transplantation and for metastatic experimentation are prepared in a similar manner. A weighed amount of viable donor tissue is passed through a Snell cytosieve with a 1:10 dilution of saline, and then using .05 cc. of the inoculum for reinjection into the recipient mouse. A more accurate evaluation of the metastatic spread from the primary tumor has been brought about by individual housing of these animals which in turn allows a longer life span. The technique of Miss Wexler in which the lungs containing the metastatic foci of non-pigmented tumor are insufflated with India ink, has continued to be a feasible method of accurately counting both the large and previously unrecognized tiny metastatic deposits on the surface of the lungs. The animals are sacrificed at weekly time intervals and respective metastatic foci counts are made and directly compared with control animals similarly sacrificed.

Major Findings:

In all tumors the trend has remained relatively consistent. By removing the primary tumor at a time when it can be completely amputated without local regrowth, these amputated animals show less metastatic tumors per mouse and per tumor mouse than the respective control animals. Attention has been particularly directed at the size of the metastatic foci found in the lungs of these animals. The justification for this emphasis arose from the repeated observations that while the number of foci was greater than in the tumor bearing control animals, the average size of the individual metastatic foci was larger than in

the respective controls. In the amputated animals the number of tumors seemed to level off at the latter sacrifice dates while the size increases. So the general observation that amputation led to the reduction of the number but an increase in the average size of the metastatic lung tumors leads to the question as to the relationship of these phenomena. Amputation could readily account for these observations if it solely reduced formation of new metastases. As a result, lung tumors occurring in animals in which the primary had been amputated would be on the average older and consequently larger than the corresponding control animals. The fact that this alone cannot account for the increased average tumor cells following amputation was demonstrated by statistical analysis of all the accounted tumors when placed into their individual logarithmically determined size categories. There is not simply a deficiency of small metastases following amputation but an actual excess of large metastases. This phenomena, while it could suggest that amputation has stimulated metastatic tumor, is interpreted as an indirect effect, reflecting either the better condition of the amputated animals or the lesser competition for nutrient material occurring in mice with a reduced number of lung metastases.

Using T-241 Sarcoma, DBA-49 Sarcoma and S-91 Melanoma a further evaluation of the tumor size has been carried out by allowing the amputated animals to go to death. A direct comparison between amputated and control animals directly depends upon the survival of the control tumor-bearing animals. Volumes of animals were used which allowed the experiment to continue after respective groups of control and amputated animals had been sacrificed at the latest date possible determined by the incipient death of the control animals. Removal of the primary tumor has allowed the T-241 bearing animals to go beyond the ten day post-amputation date when the control animals die and they may be followed up to 31 days. Animals have been sacrificed at 17, 21, 24 and 31 days and metastatic tumor counts and size analysis made. With S-91 Melanoma the latest sacrifice is 21 days post-amputation. However, amputated animals are able to be sacrificed at weekly intervals up to 12 weeks post-inoculation before the metastases causes death. DBA-49 Sarcoma studies allow 2 additional weeks of evaluation with the amputated series beyond the usual 4 week period when control animals must be sacrificed. With all three systems there has been a progressive increase in the size of the tumors. As a typical example, the S-91 Melanoma at 4 weeks had an average size of 2.3 but through the 10th week period it had gone to 9.8 average size. During this time the number of tumors per mouse and per tumor mouse had remained relatively stationary. At the latest date of sacrifice, tumor count and sizing is completely meaningless in that the tumors have coalesced and cover the greater bulk of the lungs surface. The animals in the latter stages of this experimental protocol have the same general cachectic appearance as those primary tumor bearing animals did which died some

weeks previous. It should be mentioned that repeated microscopic histological confirmation has been obtained in reference to the tiny tumors which are recognizable only by staining and three dimensional scope counting.

(b) Effect of incomplete tumor removal: Continued work has been carried out to confirm previous findings whereby the partial removal of a primary tumor has resulted in little or no change in the metastatic pattern of its dissemination. The bulk of the gross primary tumor growing in the tumor bearing leg is removed by curettement, or the tumor itself severely traumatized by massage.

Major Findings:

When the tumor is partially removed there is a rapid regrowth at the primary site and it rapidly reaches a size which is equal to the tumor size in the control animals. Additional studies with DBA-49 and S-91 Melanoma have confirmed the reported finding that partial amputated tumor bearing animals and the control tumor bearing animals have a similar metastatic pattern, both as to number and size, while the animals that have the tumor completely amputated, continue to have a fewer number of tumors but in turn a significant increase in size of the individual metastases.

(c) The effect of the age of the host: In another group of studies, mice have been aged beyond the usual 6 to 8 week period in which the studies are ordinarily carried out. Then a similar protocol is followed in an attempt to determine whether age alone will alter primary tumor growth or its metastases.

Major Findings:

In animals 6 weeks, 3 months and one year of age that were studied and compared as to metastatic behaviour, there was demonstrated a retardation of primary tumor growth in the older mice. Animals bearing, S-91 Melanoma, T-241 Sarcoma and DBA-49 Sarcoma, at one year of age had a weeks delay in growth of the tumor. This has extended the survival time of these animals as well as delaying amputation date. The trend of more tumors per mouse and per tumor mouse in control animals is persistent, with larger sized tumors being found in amputated animals.

(d) Effect of nutritional changes: Over the past year, an attempt has been made to find a percent of alcohol which the mouse will tolerate as his only source of liquid intake. In order to have prolonged life span it has been necessary to use only a 20% alcohol. The "alcoholic mice" are then placed into the metastases study protocol in

an attempt to determine any change in the primary tumor growth or alteration of metastases.

Major Findings:

By limiting the fluid intake to only a 20% concentration of alcohol, the animal at 4-5 months of age, appears active and healthy but has an approximate 10 gm. deficit in weight as compared to water fed animals. The preliminary studies have shown the primary tumor to grow slower, and to metastasize slower, but the trend continues of more metastases in the control tumor bearing animals.

(e) The effect of denervating an extremity: (Dr. Miller) The right leg was completely denervated by section of the lumbo-sacral plexus and S-91 Melanoma or Sarcoma 49 transplanted into this extremity after recovering from surgery and assurance of complete denervation. The growth rate of the primary tumor was measured and following sacrifice the number and size of pulmonary metastases was evaluated.

Major Findings:

Pilot studies with the experimental model of amputation of the primary tumor and determination of subsequent lung metastases, as well as the rate of primary tumor growth, have failed to show any alteration of the usual trends. The tumor grows somewhat slower in the thigh muscles than in controls. More metastases are found in the control animals than in those which had their primary removed, while the size of the individual foci is greater in the lungs of treated (amputated) animals. As the study progresses it is planned to show whether such factors as the milking action of muscular activity affects the behaviour of the locally implanted tumor.

(f) Effect that additional tumor systems will have: Using the experimental protocol which has been established, two mouse mammary tumors, (DBA-59 and Hoye I), and an adrenal cortical tumor are under study. The range of sacrifice dates has been altered to coincide with the rate of growth and survival time of tumor bearing animals.

Major Findings:

A mammary tumor discovered in a DBA mouse was obtained from Dr. Barrett's laboratory and is being carried in DBA mice. It has taken 12 Snell cytosieve preparations of this tumor with transplants into recipient animals before metastases has appeared. The metastatic number and size have remained consistent since the appearance of metastases. It is a rapidly growing tumor and the spread is similar to S-91 but the metastatic foci themselves are of an average smaller size than

the previously standardized tumors. A second mammary tumor, (Hoye I) found in a DBA tumor mouse in our laboratory, has taken 4 cytosieve transplantations before metastases developed. As yet the metastatic pattern has been rather inconsistent and some difficulty has been encountered in obtaining uniform primary tumor growth. An additional mammary tumor which arose spontaneously in our laboratory is under similar investigation. The adreno-cortical tumor which was obtained from Dr. Dunn's laboratory is in its preliminary stages of evaluation. Surprisingly enough we were able to obtain a consistent pattern of metastases following the second cytosieve and serial passages have failed to alter what seems to be a reliable dissemination of foci. A CD5 lymposarcoma was placed into the experimental protocol but we were unsuccessful in incorporating it into our study because of its inconsistence in development.

Pilot studies with these tumors have thus far shown a trend of metastases which favors tumor removal in order to decrease metastases.

Part II: Effect of Irradiation: (Dr. Hoye)

Methods Employed:

Cloudman S-91 Melanoma in DBA/P and in CxDBA/2F mice; the sarcoma 49 in DBA/P mice and the sarcoma T-241 in C-57 BL/6JW mice and the tumor host systems used in the study of Dr. Hoye. To 3 of 4 groups of mice, 170 r, 715 r, and 2000 r of X-ray was administered to the tumor-bearing leg alone. The growth rate of the primary tumor was compared with controls, an inoculum of the irradiated tumor was prepared and injected intravenously into mice of the same strain and then comparison made between number and size of metastatic foci which developed.

Major Findings:

The effect wich irradiation had on the growth of the "primary" tumor was manifested by an interruption in the growth curve following treatment ranging from 0-14 days. Following this delay the tumors resumed growth at a normal or near-normal rate and eventually killed all of the animals. It is to be noted that in none of the four tumor-host systems used did complete cessation of growth occur nor did tumor regression take place. The most radioresistant tumor used, sarcoma-49 in DBA mice, demonstrated no measurable response to a tumor dose of 2000 r.

The effect of irradiation on individual cells, as manifest by the number of lung metastases produced by the intravenous inoculation of the in vivo irradiated tumor cell suspension, was measured as the mean number of lung tumors per mouse from the twelve mice in each group.

This value varied considerably between the controls and each treatment group. While the best response in preventing growth of the disseminated cells was obtained following amounts of irradiation which also altered the growth curve of the "primary" tumor, this was not invariably true. The T-241 "primary" demonstrated no response to 715 r., yet no long tumors occurred following injection of its suspension, as compared to 43 tumors per mouse in control animals. The sarcoma 49 "primary" was not affected with 2000 r., yet this amount of radiation was effective in decreasing the number of lung tumors from 113 in controls to 22 tumors per mouse in the animals injected with the in vivo irradiated cell suspension.

Part III. Preferential Metastases. (Dr. Kinsey)

Methods Employed:

The Cloudman S-91 melanoma has rather consistantly metastasized only to the lungs. In order to evaluate the preference for pulmonary tissue as a metastatic site, fragments of lung removed from newborn DBA/2 mice were placed into the thigh of adult animals. Ten days later, intravascular inoculation of tumor cell suspension was carried out through the tail vein or left ventricle. All animals were then sacrificed at intervals after inoculation and a search made for metastatic implants.

Major Findings:

Following retrograde injection of the tail artery, which carries a large part of inoculum directly to the hind extremities, 37 of 40 animals developed melanotic tumors in the transplanted DBA pulmonary tissue. Inoculation bia the left ventricle produced artificial metastatic lesions in the grafted DBA pulmonary tissue in 4 of 8 animals so treated, suggesting that there becomes less chance of embolic cells reaching the transplanted tissue after this route of administration. Surprisingly 2 of 8 animals developed tumors in the transplanted lung after tail vein inoculation. Some intravascular cells had escaped the in situ pulmonary capillary network and had selected the grafted lung to implant.

In no other transplanted tissue were artificial metastatic lesions found, and all animals in the experiment had tumor implants in the in situ lung regardless of the route of administration of the tumor suspension.

Part IV. Relationship of Circulating Tumor Cells and Metastases:

Methods Employed:

A suspension of Sarcoma T-241 tumor was injected into the hind leg of C-57 mice. a) Daily amputation of the tumor bearing extremities were carried out and then the animals sacrificed and observations made as to the presence of pulmonary metastases. b) A second group were sacrificed at daily intervals after tumor inoculation in the thigh, their lungs ground into an emulsion and this was injected into the peritoneal cavity of normal mice. These animals were then followed for the development of abdominal tumor. c) On successive days after thigh inoculation .06 to 1.0 cc. of blood was drawn from the heart and processed by the Dextran-Millipore filter technique for receiving of tumor cells.

Major Findings:

A) If thigh amputation was done before the 7th day after inoculation, pulmonary foci of metastases were not seen. After 7 days, the incidence of pulmonary metastases increased. B) If amputation was carried out before the 7th day, the biological tests for pulmonary metastases were negative. Amputation delay beyond 7 days resulted in progressive increase in the proportion of animals with positive biological tests for pulmonary metastases as indicated by the intra-abdominal growth of tumor. C) Tumor cells were found in the heart blood as early as 24 hours after thigh inoculation with sarcoma T-241 and thereafter increasingly greater numbers of tumor cells were found in the blood.

The demonstration of tumor cells in the blood is not equivalent to the development of hematogenous metastases and suggests that some quantitative effect of the number of circulating tumor cells is involved before hematogenous foci will develop.

Part V: The Effect of a Foreign Body on Metastases and Tumor Spread Upon the Foreign Body.

Methods Employed:

Etheron sponge is basically produced from di-isocyanate polyethers and contains millions of uniformly distributed inter-connected air-cells. From one large block of sponge, 7 mm. cubes were cut for use in all experiments. These were implanted subcutaneously on the backs of the animals. Animals employed in all phases of this study were DBA/P gray mice. Three transplantable tumors with distinctly different characteristics were utilized:

1) mast cell tumor which metastasizes to liver and spleen via the blood stream and is lethal in about 13 days;

2) an L-1210 leukemia variant (J325-4B10) which is infiltrating and widely disseminated; and 3) S-91 melanoma which metastasizes to the lungs at about 5 weeks. These tumors were inoculated in different groups either in the leg at a site distant from the sponge or in the back adjacent to the sponge. In another group of mice, these tumors were placed in a depression formed in the sponge which was then inserted tumor side down on the backs of the animals. The histology of implanted sponges was determined at intervals from 6 hours to 5 months.

Major Findings:

Etheron did not function as a matrix for local tumor growth and resisted direct tumor invasion into its framework. Tumor growth was by circumferential spread. Metastases from the three transplantable tumors to distantly embedded Etheron did not occur. When Etheron was implanted locally with each of the three tumors or at a site distant from them, it did not alter their take, latency, weight, or metastatic pattern. Histologically, Etheron sponge evoked a chronic inflammatory and foreign body response and eventually became fragmented and replaced by firm connective tissue. Plastic implants are inserted frequently now by surgeons, especially plastic surgeons. Conclusive animal experimentation has shown that all plastics so far tested induce sarcomas in varying frequency. Two other cancer related problems also arise. With current stress on circulating tumor cells and dormant tumor cells, a porous foreign body might function as an artificial stroma on which such tumor emboli could implant and grow. In addition, the insertion of a spongy foreign body into an area that previously contained tumor might influence the development of future would recurrence by irritation or by providing a matrix for growth. Functioning as a foreign body and irritant in this experiment, Etheron sponge had no effect on the characteristics and behavior of three different laboratory tumors.

Significance to Cancer Research:

By the use of standardized tumor-host systems it is anticipated that a more thorough understanding of the different factors affecting metastases might be brought to our attention. If human cancer and its therapy is to be understood and improved, an understanding of the factors that control metastases must be found. Amputation, nutrition, massage, and irradiation all are factors which are applicable to the over all understanding of the causes and failures of cancer therapy.

Proposed Course of Project:

The present studies will be continued and their scope of value to cancer understanding, widened. In the laboratory model it is hoped that we can determine the effect upon tumor growth and its metastases of

numerous intrinsic as well as extrinsic factors. Stress, trauma, irradiation, infection, nutritional factors and hormonal imbalance are all to be further studied.

It is proposed to expand the present study into the relationship of circulating tumor cells and the incidence of metastatic tumor occurrence. If a correlation between the two can be shown or fails to be shown, this in itself will open further facets of investigation.

Part B included Yes /XX/ No /__/

NCI-10-759(c)
Serial No.

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Hoye, Robert C., and Smith, Robert R.: The Effectiveness of Small Amounts of Irradiation in Decreasing the Number of Metastases in an Experimental System. Surgical Forum. Vol. X, 72-77, 1960.

Hoye, Robert C., and Smith, Robert R.: The Effectiveness of Small Amounts of Preoperative Irradiation in Preventing the Growth of Tumor Cells Disseminated at Surgery. An Experimental Study. (Accepted for publication in Cancer).

Kinsey, David L.: An Experimental Study of Preferential Metastasis. Cancer. 13:4, 674-676, 1960.

Ketcham, Alfred S., Kinsey, David L., Wexler, Hilda, and Mantel, Nathan: The Development of Spontaneous Metastases after the Removal of a "Primary" Tumor. II. Standardization Protocol of Five Animal Tumors. (Accepted for publication in Cancer).

Serial No. NCI-10-762(c)
1. Surgery Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Study of Metabolic Changes Associated with Cancer Surgery.

Principal Investigators: Dr. Alfred Ketcham, Dr. Edward McLaughlin, Dr. Howard Radwin, and Dr. Jack Bloch

Other Investigators:

Cooperating Units: Dr. Richard Malmgren, Path. Anatomy, Dr. Milton Winitz, Lab. Biochemistry, NCI, Dr. Nathaniel Berlin, Gen. Med. Br., NCI, Dr. Julius White, and Florence Millar, Lab. Physiology, NCI.

Man Years (calendar year 1960):
Total: 1½
Professional: 1¼
Other: ¼

Patient Days (calendar year 1960):

Project Description:

Part 1. Total Red Cell Volume Changes Associated with Radical Surgery. (Dr. Ketcham)

Part 2. Effect of Synthetic Water Soluble Diet on Post-traumatic Nitrogen Balance. (Dr. Radwin)

Part 3. Mitotic Stimulating Effect of Serum from Patients with Cancer. (Dr. McLaughlin)

Part 4. Water Exchange in Animal and Human Tumors. (Dr. Bloch)

Objectives:

Part 1. There are many reports in the literature dealing with the measurement and replacement of blood loss during surgery, but few have dealt with the changes in blood volume which may occur in the post-operative period. The present study was designed to measure the loss of red blood cells in patients undergoing extensive surgery, during both the operation and the postoperative phase. It was further designed to demonstrate blood loss which is not usually measured in the operating room and to introduce a simple method for its determination.

Methods Employed:

Total red cell volume determination using Na2, Cr5104 were carried out immediately preoperatively, immediately postoperatively, and on the second and fourteenth postoperative days. A modification of the technique as described by Reed was used. The samples were counted in a well type scintillation counter using a single channel gamma ray spectrometer. All samples were measured to a counting error of plus or minus 1%. The entire technique as used in this study gave an average difference of 1.5% with a range of 0-4% in 5 control subjects having duplicate determinations several days apart. The turnover or loss of red blood cells was calculated from the difference in the two red blood cell volume determinations plus 200 cc. per unit of blood per each transfusion given. For the purpose of comparison, the total red cell volume loss has been converted to whole blood loss by dividing by the average hematocrit during the interval studied. Blood lost at surgery was measured in the electronic blood loss meter described by La Veen and Rubricus. In addition to the measurement of blood on the sponges, laparotomy pads, towels, and via suction, all the drapes and gowns were measured for blood content in the latter part of the study. It was shown that 200 cc. of whole blood poured onto a surgical drape would record a loss of 200 cc. when the blood-stained portion of the drape was placed into the machine for 3 to 5 minutes of manual agitation. The patients were selected at random from a series of patients scheduled to undergo radical surgery. The normal total red cell volume for each patient was calculated from the patient's average stated normal weight and using 30 cc. per kilogram for males and 25 cc. kilogram for females.

Major Findings:

The subsequent tables 1,2,3 list the data obtained by measurement and calculation for each of the patients in the study. A comparison of the immediate preoperative and immediate postoperative total red cell volume shows that in 10 of the 16 patients studied, there was a failure to replace the blood loss at surgery varying up to 794 cc. of red cells. In three instances there was in addition to replacement a net gain up to 256 cc. and in 3 instances no significant changes occurred. The total red cell volume turnover varied from 576 cc. to 339 cc. This loss has also been shown in terms of cc. per kilogram of body weight so that a comparison can be made of the total red cell loss without consideration of differences in body weight. Table 2 shows the blood loss in the drapes and gowns to range from 350 to 1465 cc. and indicates the necessity of including blood loss on drapes and gowns in the determination of operative blood loss. In 9 patients, when only the laparotomy pads, sponges, and suction were considered, there was a 371 to a 2700 cc. deficit in unreplaced whole blood. In the remaining 7 patients the drapes and gowns were also measured and the unreplaced blood loss varied from a -464 cc. to +24 cc. of whole blood. The loss measured in drapes and gowns accounted for 1/7 to 1/3 of the total blood loss. Table 3

shows that the loss of red blood cells in the postoperative period varied from 160 cc. to 477 cc. In the last column of Table 3 this additional loss is compared with the total red cell volume immediately postoperatively and represents a 9% to 44% decrease in total circulating red cell volume. 14 of the 16 patients were anemic by blood volume standards by the 14 postoperative day. This is demonstrated by comparing the preoperative total red cell volumes in Table 1 with the 14 day total red cell volumes seen for each patient in Table 3.

The hematocrit was an unreliable guide in assessing changes in the patients blood volume. In Table 4, 6 of the 11 patients who were not transfused postoperatively are represented. An average red cell loss of 350 cc. in the 3 patients over the 14 day postoperative period changed their hematocrit only an average of 5, yet 3 other patients having an average loss of 209 cc. of cells had an average hematocrit change of 13.

In the patients in this study the total red cell volume on admission was not determined and the peripheral blood counts were used as indicators for transfusion need preoperatively. This was reflected in the immediate preoperative total red cell volume determination which was as much as 38% less than the calculated normal.

The value of knowing the total loss as it occurs in the operating room is obvious in patients losing from 5 to 20 units of blood during a 4 to 8 hour surgical procedure. With the use of the blood loss meter the measurement of this loss is simple and accurate if the gowns and drapes are measured. In the 7 patients selected at random, the blood on the drapes was considerably in excess of the loss as estimated by the surgeon and comprised from 15% to 42% of the total operative loss.

Of equal significance to the loss which occurs in the operating room is the loss which occurs postoperatively. This loss occurs insidiously and will frequently remain unrecognized unless total red cell volume is measured. The loss has varied from 9% to 44%, and averaged 22% of the immediate postoperative total red cell volume. Part of this loss undoubtedly is due to the continued seepage of blood at the drain site but other processes must be responsible.

There are 3 rather conclusive results to this study. The first being that from 15% to 42% of the surgical blood loss is on the drapes and gowns. Secondly, that following radical surgery the insidious loss of red cells in the postoperative period may equal the blood loss and in this study it represented 9% to 44% of the total red cell volume present immediately postoperatively. Thirdly, the postoperative hematocrit is a convenient but an unreliable guide in assessing the further loss of red cells in the operative period.

Significance to Cancer Research:

The surgical exploration of cancer has been one of the chief projects of the Surgery Branch. The blood volume studies more adequately allow the surgeon to prepare his patient for surgery as well as to evaluate some of the metabolic changes which are in evidence during the patient's postoperative convalescence. In carrying out extensive surgery it is essential that the procedure be performed under the most optimum of conditions and also followed postoperatively to prevent the development of any major complications. It has been apparent that the development of one complication in these patients has usually led to a series of difficulties increasing the morbidity and mortality.

Proposed Course of Project:

Over the past few months, catheter aspiration of postoperative wound drainage has been carried out. The amount of serous material has been collected and measured over the course of the patient's convalescence. It is hoped that by utilizing additional radioisotopic techniques this wound drainage material will allow, through isotopic dilution, the total day-to-day red cell loss determination. This loss can be compared to the loss as determined by decrease in total red cell mass.

Part 2. Effect of Synthetic Water Soluble Diet on Post-traumatic Nitrogen Balance. (Dr. Radwin)

Objectives:

Major exenterative surgery, such as is performed by the Surgery Branch of the Cancer Institute, is disruptive to the general metabolism of the patient, and recovery is a burden to the reserves of those who are frequently already debilitated. The demands of convalescence might be eased if the patient were provided with the proper nutrients in the postoperative period. However, this would depend on the ability of the patient to utilize these nutrients. In this study a water soluble, synthetic, chemically defined diet developed at NIH is employed to discover in quantitative terms the value of a complete diet to the injured animal. Growth rate is followed as a measurable parameter of total body metabolism, and nitrogen balances are determined.

Methods Employed:

Forty male Sprague-Dawley white rats were the experimental animals. They were subjected to varying dietary regimens both in quantity and in the method of feeding, some being force-fed and others permitted to ingest ad libitum. The experimental group was subjected to a crush injury to the femur producing a fracture on day 5 and were then further observed for nine postoperative days. Nitrogen balances and body weight were determined daily. At the conclusion of the experiment carcass analyses were performed on some animals.

Major Findings:

The effect of injury on growth rate is influenced by the nutritional status of the animal. When the growth rates of injured and uninjured animals on 18 cc. diet are compared, a marked lag in growth of the former is observed, while difference in those receiving 24 cc. is considerably less in extent and duration. The data on nitrogen balance support the hypothesis that the injured animal can utilize balanced dietary supplements for growth.

A deleterious effect of injury on growth and nitrogen balance was observed. However, provision of adequate nutrients after trauma appears to be worthwhile in that much of the decrease in growth rate and nitrogen retention can be prevented.

Significance to Cancer Research:

The value of adequate replacement of protein in the postoperative patient needs to be determined. The increasing scope of radical surgery requires study of means of nitrogen replacement.

Proposed Course of Project:

Project is complete and results published.

Part 3. Mitotic Stimulating Effect of Serum from Patients with Cancer. (Dr. McLaughlin)

Objectives:

To determine if there is a difference between serum from patients with cancer and serum from patients without cancer as measured by their ability to stimulate mitosis in a biological test system.

Methods Employed:

The biological test system utilized is the white rat after a partial hepatectomy has been performed.

Female Sprague-Dawley strain white rats weighing 170-200 grams are used. Partial hepatectomy is performed by excising the left lateral and median lobes of the liver. Seventy-two hours post-partial hepatectomy, the animals are sacrificed. Those animals that are to receive injections of serum are given one milliliter intraperitioneally every eight hours during the seventy-two hours of the experiment.

The rate of mitotic activity was determined by three methods: 1) Dry weights of the liver. 2) Mitotic counts of sections of liver tissue. 3) Amount of radioactive DNA synthesized during the test period.

When dry weights of the liver were employed, the increase in the size of the hepatic segment during the course of the experiment is reported as "percent liver regeneration."

When mitotic counts of liver sections are employed, five thousand cells are scanned, and those cells in anaphase or metaphase are counted. The increase in mitotic activity is reported as "mitotic counts per 5,000 cells."

When the amount of radioactive DNA is measured, those animals to be evaluated receive one microcurie of Tritium labelled thymidine every hour for twenty-four consecutive hours, beginning with the sixth and ending with the thirtieth hour post-partial hepatectomy. The liver DNA is extracted and measured, the DNA is then placed in a liquid oscillation counter and the amount of DNA synthesized is measured and recorded as "counts per minute per 100 micrograms of DNA."

Patient Material:

Serum is obtained from patients of the Surgery Branch for evaluation by all three methods of measuring mitotic activity.

Major Findings:

When the three methods of evaluation are used in parallel experiments, it can be shown that all reflect the ability of serum from patients with cancer to stimulate mitosis in the liver remnant when compared to serum from patients without cancer.

When mitotic counts of liver sections are made, it is demonstrated that cancer serum produces twice the number of mitotic counts as does serum from patients without cancer.

Approximately twice as much radioactive DNA is synthesized by those animals receiving serum from patients with cancer as compared to animals receiving serum from patients without cancer.

By dry weight determinations approximately 50% of the liver regenerates when serum from patients without cancer is injected while in excess of 70% of the liver regenerates when serum from patients with cancer is injected.

Thus, any of the three methods may be used to evaluate serum and to measure stimulation or inhibition of mitotic activity in the test system, because any one method reflects what would occur if either of the other methods were utilized.

Studies have shown that serum from patients with cancer will

stimulate mitotic activity and liver regeneration in this biological test system, while serum from patients who do not have cancer will inhibit regeneration of the liver.

The nutritional status of the serum donor does not interfere with the expected stimulating or inhibitory mitotic effect of the serum. Animal studies have shown that starved animals inhibit mitotic activity as do well nourished serum donors.

Animals with a transplanted tumor will inhibit liver regeneration while serum from animals with a spontaneous tumor will stimulate liver regeneration, which suggests that the changes in the serum which are being measured are present only with spontaneous tumors.

The site of the primary cancer does not seem to influence the mitotic stimulating effect of the serum. Serum samples, obtained from patients whose primary lesion was located at several different sites stimulated mitotic activity to a comparable degree.

Previous treatment did not seem to influence the mitotic stimulating effect of the serum. A group of patients, who prior to admission had been treated with radiation, with surgery, or with radiation and surgery, were evaluated and no significant difference in their mitotic stimulating capacity could be demonstrated.

Excision of the primary lesion by radical surgical techniques did not alter the mitotic stimulating effect of the serum of patients with cancer. Serum from a group of patients with cancer of the cervix obtained prior to pelvic exenteration, and six and twelve weeks post-operatively, was evaluated. No significant difference in the mitotic stimulating function of the various serum samples could be demonstrated.

Significance to Cancer Research:

Numerous reports of new primary cancer developing after the removal of a previous primary lesion, autopsy studies which show multiple sites of cancer of different cell types and in different organ systems, and the different clinical courses followed by a group of patients with the same site and cell type of cancer, give rise to the speculation that there may be alterations in the extracellular fluid of cancer patients, and that these alterations may be reflected in the serum of patients with cancer. This study has demonstrated that when a specific biological test system is used, one can demonstrate that serum from patients with cancer does stimulate mitotic activity when compared to the effects of serum from patients who do not have cancer.

Proposed Course of Project:

Studies are in progress to evaluate the effect of various serum

fractions upon hepatic regeneration following partial hepatectomy.

Part 4. Water Exchange in Animal and Human Tumors. (Dr. Bloch)

Objectives:

To determine and compare the rates at which different animal and human tumors reach equilibrium with tritium labelled water in the plasma after intravenous administration of HTO.

Methods Employed:

Animal Experiments: Tritiated water (HTO) in dosage of 100 mc. in 0.2 cc. is injected into the tail veins of 200-250 gram male Sprague-Dawley rats bearing a two to four week old Walker 256 carcinosarcoma subcutaneously on the back. Animals are sacrificed by intracardiac exsanguinating puncture at serial time intervals ranging from 5 minutes to 12 hours. Samples of blood, liver, muscle, tumor, and skin are lyophilized, and the water collected is measured for specific activity of tritium in the Packard Liquid Scintillation Counter. The ratio of tissue or tumor water/plasma water is recorded.

Human Studies: Tritiated water (HTO) in dosage of 200 mc. in 1 cc. is intravenously injected into human subjects with tumor, following drawing of a base-line blood sample. Following injection, serial blood samples are taken. Tumor is removed and time of excision during operative procedure recorded. Tumor and blood are lyophilized and the water extracted analyzed for tritium activity in the same manner as the previously described animal samples.

Major Findings:

The equilibration of tritiated water in the blood with the water of 8 tumors of wide histologic variety and anatomic location in 4 different host species, rat, mouse, hamster, and man, was studied. Evidence was obtained that the rate of water exchange for 7 of these tumors is essentially the same; that tumor water in comparison with liver, muscle, and skin, equilibrates slowly with blood water; that the major limiting factor in this exchange is blood supply; and that the rate of water exchange in the tumor is increased by ether anesthesia.

Significance to Cancer Research:

The demonstration that the water molecule equilibrates more slowly with tumor than with any other body tissues studied points out an important consideration in drug therapy of cancer. Independent of other biochemical features, if a systemically administered drug is taken up more rapidly and preferentially by other body tissue, it may not

reach the tumor in sufficient quantity to be effective.

Proposed Course of Project:

The study is complete and publication of results will be out in a few months.

Part B included Yes /XX/ No /__/

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Radwin, Howard M., Rechcigl, M., and Greenstein, Jesse P.: The Effect of Diet on Growth and Nitrogen Balance After Injury. Surgery. 48:4, 687-697, 1960.

Bloch, Jack H., Millar, Florence K., Harris, Alvin R., Berlin, Nathaniel I. and White, Julius.: Water Exchange in Animal and Human Tumors. (Accepted for publication in Journal of Applied Physiology).

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TABLE I

TOTAL RED CELL VOLUME

IMMEDIATELY PRE- AND POST-OPERATIVELY

Pt.	Sex	Age	Operation	TRCV		RBC's replaced at surg.*	TRCV Post-op	Turnover at Surg.	
				Calculated Normal	Pre-op			Total RBC	RBC cc/kg.
H.C.	F	48	Total Pelvic Exenteration	1450cc	1559cc	1000cc	1347cc	1212cc	20.9
R.M.	F	52	Total Pelvic Exenteration	1450cc	1396cc	800cc	1166cc	1030cc	17.8
W.S.	F	64	Total Pelvic Exenteration	1550cc	1288cc	1400cc	1544cc	1144cc	18.5
C.H.	M	48	Laryngectomy Rad. Neck	1200cc	1257cc	1000cc	1114cc	1143cc	28.6
A.B.	F	37	Radical Hysterectomy	1350cc	1603cc	1400cc	1485cc	1518cc	28.1
E.R.	F	46	Ant. Pelvic Exenteration	1850cc	1163cc	1200cc	1411cc	952cc	12.9
T.M.	F	56	Ant. Pelvic Exenteration	1575cc	982cc	2600cc	817cc	2765cc	43.9
D.A.	F	44	Ant. Pelvic Exenteration	1725cc	1828cc	1400cc	1206cc	2022cc	29.3
O.T.	M	64	Rad. Excision Pelvic Tumor	2730cc	2487cc	2600cc	1693cc	3394cc	37.3
M.D.	M	41	Parotidectomy Rad. Neck	2370cc	1509cc	1200cc	1360cc	1149cc	14.5
C.F.	M	49	Laryngectomy Rad. Neck	2310cc	1967cc	600cc	1991cc	576cc	7.5
M.H.	F	51	Post.Pelvic Exenteration	1200cc	1306cc	1400cc	1097cc	1609cc	33.5
R.R.	F	39	Radical Hysterectomy	1500cc	1292cc	1800cc	1352cc	1740cc	29.0
C.F.	F	62	Mandibulectomy Rad. Neck	1375cc	1619cc	2000cc	1117cc	2502cc	45.5
J.P.	M	64	Hemi. Pelvectomy	1728cc	1506cc	1200cc	1541cc	1165cc	20.1
S.M.	F	34	Radical Hysterectomy	1600cc	1319cc	800cc	1308cc	811cc	12.7

* 200cc RBC contained in 500cc whole blood

TABLE II

OPERATIVE WHOLE BLOOD LOSS

Patient	Measured Loss* Suction Sponges cc.	Gowns Drapes cc.	Measured Loss Blood Volume Determinations cc.	Deficit in** Unreplaced Whole Blood cc.	% of Total Loss
H.C.	1700	(·)	3030	-1330	44%
R.M.	1650	(·)	2341	- 691	30%
W.S.	2200	(·)	2571	- 371	14%
C.H.	2200	(·)	3307	-1107	33%
A.B.	2800	(·)	3750	- 950	25%
E.R.	2000	(·)	2375	- 375	16%
T.M.	6000	(·)	7900	-1900	24%
D.A.	3700	(·)	4878	-1178	24%
O.T.	4900	(·)	7600	-2700	36%
M.D.	2100	1050	2985	+ 165	5%
C.F.	950	350	1369	- 69	5%
M.H.	2200	1150	3575	- 225	6%
R.R.	3800	645	4193	+ 252	6%
C.F.	4100	1465	6029	- 464	8%
J.P.	1800	1285	2949	+ 136	5%
S.M.	1500	655	2205	- 50	2%

* Measured on Automatic Conductivity Blood Loss Meter.
(·) This loss was not measured in the first nine patients studied.
*** Error in measured loss is the difference between loss measured from TRCV determinations and blood measured in suction, sponges, etc.
Column III-(Col. I & II) = Column IV.

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TABLE III

RED BLOOD CELLS LOST IN THE POSTOPERATIVE PERIOD

Pt.	Postoperative TRCV			CC of RBC Transfused Post-op	RBC Loss		Total	Post-op* TRCV Loss in 2 weeks
	Immed.	Day 2	Day 14		First 2 days	Day 2-14		
H.C.	1347cc	1095cc	1035cc	0cc	250cc	62cc	312cc	23%
R.M.	1166cc	1116cc	1000cc	0cc	50cc	116cc	166cc	14%
W.S.	1544cc	1276cc	1099cc	0cc	268cc	177cc	445cc	29%
C.H.	1114cc	901cc	919cc	0cc	213cc	18cc	195cc	18%
A.B.	1485cc	1448cc	1266cc	0cc	40cc	179cc	219cc	15%
E.R.	1411cc		1144cc	0cc			267cc	19%
T.M.	817cc	866cc	1056cc	600cc	151cc	210cc	361cc	44%
D.A.	1206cc	1257cc	1170cc	400cc	350cc	86cc	436cc	36%
O.T.	1693cc		1732cc	200cc			160cc	9%
M.D.	1360cc		1330cc	200cc			230cc	17%
C.F.	1991cc		1640cc	0cc			351cc	18%
M.H.	1097cc	898cc	694cc	0cc	200cc	203cc	403cc	37%
R.R.	1352cc	1321cc	1070cc	0cc	31cc	251cc	282cc	21%
C.F.	1117cc	1014cc	930cc	0cc	103cc	84cc	187cc	17%
J.P.	1541cc	1300cc	1227cc	0cc	241cc	73cc	314cc	20%
S.M.	1308cc	965cc	931cc	100cc	423cc	54cc	477cc	36%

* RBC Loss x 100 Postop. TRCV

TABLE IV

THE FALLACY OF RELYING ON THE POST-OP HcT

Pt.	Normal TRCV	Operation	Immediately Post-op		14th Day Post-op		Total 14 Day Change in	
			TRCV	HcT	TRCV	HcT	TRCV	HcT
W.S.	1550cc	Total Exenteration	1544cc	49	1099cc	43	445cc	6
J.P.	1728cc	Hemipelvectomy	1541cc	40	1227cc	35	314cc	5
C.F.	2310cc	Larynx-Neck	1991cc	43	1640cc	39	351cc	4
Av.=	1863cc		1692cc		1322cc		370cc	5
R.M.	1450cc	Total Exenteration	1166cc	46	1000cc	31	166cc	15
E.R.	1850cc	Ant. Exenteration	1411cc	46	1144cc	32	267cc	14
C.H.	1200cc	Larynx-Neck	1114cc	42	919cc	32	195cc	10
Av.=	1500cc		1230cc		1021cc		209cc	13

Serial No. NCI-10-765
1. Surgery Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Effect of Carcinogenic Agents Using a Bronchial Pouch Preparation in the Dog.

Principal Investigator: Dr. Alfred S. Ketcham

Other Investigators: Dr. W. C. Hueper and Dr. William Banfield

Cooperating Units: None

Man Years (calendar year 1960):
Total: ½
Professional: ½
Other: 0

Patient Days (calendar year 1960):

Project Description:

Objectives:

1. To introduce and utilize the blind pouch technique into bronchogenic carcinoma carcinogenesis study. Results may be added to current evidence concerning the etiology of human lung cancer.

2. To provide a satisfactory large animal tumor for treatment studies.

Methods Employed:

As performed at present, the pouch is fashioned from the left lower lobar bronchus. A left lobectomy is first performed, amputating the bronchus more distal than usual. The lower lobe bronchus is then re-amputated at a higher level, severing the cartilagenous ring and endobronchial structures, but leaving the peribronchial structures intact as a vascular pedicle. The left main bronchus is sutured as are both ends of the pouch, forming in effect a pseudocyst. The end of a fine polyethylene catheter is implanted into the lumen of this pouch, and the other end of the catheter is exteriorized through the chest wall where it is buried beneath the skin, available for injection purposes.

Suspected carcinogenic agents will be injected into the bronchial pouch via the polyethylene catheter at regular intervals. Suitable

controls where the vehicle only is injected will be included. Serial roentgenograms will be taken to detect and follow the progress of any left hilar radiodensity which may develop. The animals will be followed for four to five years, after which time they will be sacrificed. It is proposed to study the following agents initially: 3,4 benzpyrene, tobacco tar, and internal combustion engine exhaust concentrate. These three compounds are being injected into different animals, and a fourth group of control animals are being observed.

Major Findings:

This project was instituted and carried on by Dr. John Waite until his transfer to another Public Health Service Hospital. It is being completed by Dr. Alfred S. Ketcham, Surgery Branch, NCI, and Dr. William Banfield is doing the microscopic evaluation of the pouches.

The animals have been followed at three-month intervals with re-injection of tobacco tar into their bronchial pouches, and clinical evaluation of all animals within the experiment, which includes chest x-ray evaluation. This is intended to be carried out throughout the course of the study which extends over the next two years. The 1960 x-ray evaluation of the remaining dogs failed to show any significant findings. Four animals have died within the past year and two have been sacrificed. The pathological specimens, both grossly and microscopically, have failed to show any evidence of cancer. There are presently 16 dogs remaining in the study. One animal which recently died has gross evidence of tumor, but this has not been microscopically confirmed at this time. This animal was in the tobacco tar series.

Significance to Cancer Research:

To date the majority of evidence purporting to bear on the genesis of human bronchogenic carcinoma is adduced from the ability of a suspected carcinogenic agent to produce a tumor when painted on the skin of a rabbit or mouse. In interpreting the results of these experiments, not only must the heterogenicity of the experimental animal with respect to humans be considered, but dissimilarity between epidermis and bronchial epithelium plays an important role. Exposing the experimental animal to an atmosphere laden with the suspected carcinogenic agent has almost always been unsuccessful in producing tumors. This had led to the conclusion by some investigators that the agents are not respiratory carcinogens and by others that the animal's respiratory defenses (the cough, the dilution of the carcinogenic agent in the respiratory secretions, and the action of cilia) are so efficient as to make exposure to the carcinogenic agent negligible using this technique. There is much logic to support the second hypothesis, and a good method to provide contact with a strong concentration of the suspected agent over a prolonged period of time and against which contact the animal may not have an effective defensive mechanism might be of some value in settling this point. We feel that a

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study on the uptake of radioactive benzpyrene from our pouch preparation alone might yield sufficient data to justify the project.

Proposed Course of Project:

This project is being carried on by Dr. Alfred Ketcham, Surgery Branch, with the assistance of Dr. Banfield. The animals are evaluated at three month intervals, but due to inability to obtain the tobacco tar from Dr. Hueper, re-injection of the one remaining patent polyethylene tube has not been carried out within the past 16 months. During the next year animals will be intermittently sacrificed as the protocol calls for and the study will terminate in the Spring of 1961.

Part B included Yes / / No /X/

Serial No. NCI-10-773
1. Surgery Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Study of Host Factors Associated with Implantation and Growth of Cancer Cells.

Principal Investigators: Dr. Marvin Arons, Dr. Donald Morton, and Dr. Alan Retik

Other Investigators: Dr. Alfred Ketcham, Dr. Robert Smith, Dr. Seymour Nash, and Mr. Nathan Mantel

Man Years (calendar year 1960):
Total: 2
Professional: 1¼
Other: 3/4

Patient Days (calendar year 1960):

Part 1. Effect of Growth Hormone, ACTH, Cortisone, and Amputation of Tumor Bearing Extremity on a Transplanted Tumor. (Dr. Arons)

Part 2. The Heterologous Transplantation of Human Cancer Tissue into Normal Rats, and Growth in Millipore Chambers. (Dr. Morton)

Part 3. The Effect of Experimental Environmental Factors, especially Hepatic Trauma, on the Development of Tumor Metastases. (Dr. Retik)

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Part 1.

Project Title: Effect of Growth Hormone, ACTH, Cortisone, and Amputation of Tumor Bearing Extremity on a Transplanted Tumor and Its Metastases.

Principal Investigator: Dr. Marvin Arons

Other Investigators: Dr. Alfred Ketcham, Dr. Seymour Sabesin, Mr. Nathan Mantel, and Miss Hilda Wexler

Cooperating Units: Biometry Branch, NCI and Pathologic Anatomy Branch, NCI

Project Description:

Objectives:

By employing a previously standardized tumor-host system, this study was designed to determine the effect of growth hormone and ACTH on primary and metastatic tumor growth and to study the behavior of a tumor previously hormonally treated and then transplanted.

By employing a previously standardized tumor-host system, additional experiments underway are designed to determine:

1) The effect of cortisone on the incidence of metastases;

2) The effect of primary tumor amputation and adjunctive cortisone therapy on the incidence of metastases;

3) The effect of cortisone on altering the anatomical pattern of metastases;

4) The effect of cortisone on primary tumor growth.

Methods Employed:

The DBA-49 sarcoma was inoculated into groups of DBA/P mice. The first group received injections of H_2O, the second growth hormone, the third ACTH, the fourth growth hormone and ACTH, the fifth inactivated growth hormone, and the sixth group received neither tumor not drug. Until the experiments ended on day 21, the primary tumors were sized every 3 days and weighed at the time of sacrifice. The initial dose of growth hormone was .5 mg for the first 6 days. It was then increased by .5 mg every 6 days. The initial dose of ACTH was .05 mg for the first 12 days and then .08 mg thereafter. At the time of sacrifice and examination of the lungs for metastases, the primary tumors were transferred to other groups of corresponding animals, none of which received any hormone therapy.

In the second part of this study, T-241 sarcoma which is known to spontaneously metastasize 6 days after transplantation was inoculated into 15 groups of C-57 black mice. The daily dosage of cortisone acetate was 1 mg intraperitoneally. The groups were arranged so that some received 3 days of pre-inoculation cortisone therapy, some 3 days of pre-treatment combined with 12 days of post-tumor inoculation therapy, others 12 days of post-treatment alone, and still others a delayed 6 day post-cortisone regime. Primary tumors either remained intact or were amputated 6 days or 8 days after their initial transplantation. One of these groups served as a control without hormone injection. A sixth group functioned as a control without tumor or drug therapy. At sacrifice on day 21, primary tumors were weighed, lungs examined grossly and livers, kidneys, and spleens microscopically for metastases.

Major Findings:

It was found that growth hormone simultaneously increased primary tumor growth, carcass weight, and the number of large size pulmonary metastases. Though this may have been a secondary nutritional effect, it did not result from any antigenic component of growth hormone. ACTH simultaneously decreased tumor growth, carcass weight, and the number of large size lung tumors. When ACTH and growth hormone were administered conjunctively, only the effects of growth hormone were observed. Both growth hormone and ACTH appeared to have an antagonistic action. Neither growth hormone nor ACTH altered tumor take, latency, or growth rate of the primary tumor. Finally, the effect of growth hormone and ACTH on the primary and secondary tumors was not transferred when the primary tumor was transplanted to untreated animals.

The study designed to measure the effect of cortisone and amputation of tumor on metastases has been completed, but data has not yet been compiled.

Significance to Cancer Research:

The basic regulatory mechanisms which influence metastases continue to be poorly understood. Despite many experiments with adrenal steroids, especially cortisone, many of the conclusions are contradictory regarding site of action, effect, and underlying cause. This suggested the need for further investigation of some probably underlying process common to all the variable results recorded. It is hoped that the design of this experiment will contribute to the knowledge of the metastatic process.

By using a standardized and nearly isologous tumor-host system, these results shed more light on the basic regulatory mechanism which influence primary tumor growth and metastases.

NCI-10-773
Serial No.
Part 2.

Project Title: The Heterologous Transplantation of Human Cancer Tissue into Normal Rats, and Growth in Millipore Chambers.

Principal Investigators: Dr. Donald Morton, and Dr. Seymour Nash

Project Description:

Objectives:

1. The successful transplantation of human cancer tissue into normal rats using the principle of acquired immunological tolerance.

2. The use of these human cancer transplants to investigate the response of human tumors to various local and systemic chemotherapeutic agents.

3. The use of the Millipore chamber technique to grow human tumor cells in laboratory animals.

4. The use of this technique to determine the sensitivity of individual tumor cells to various chemotherapeutic agents and cytotoxic antisera.

Methods Employed:

The phenomena of acquired immunological tolerance is based upon the fact that a newborn rat will not form antibodies to an antigen if it has been exposed to that antigen before immunological maturity is achieved. The destruction of tumor heterografts is mediated through an immunological process. Therefore, newborn rats will be injected with human blood within 24 hours from birth and will receive repeated injections over the next two to three weeks to establish tolerance to human tissues. These rats will then receive transplants of human tumor tissues. If successful transplantation is accomplished, a new tumor line will be established by serial transplantation which can be used for various local and systemic chemotherapeutic studies.

Millipore chambers with pores .45 microns in size will permit the free diffusion of oxygen, carbon dioxide and nutrient substances across the membrane, but will block the passage of cells. Destruction of a foreign tumor graft depends upon direct contact of host lymphocytes with the tumor cells. Therefore, isolation of human tumor cells in a Millipore chamber placed in laboratory animals should prevent their destruction and allow them to grow and multiply. The effects of various chemotherapeutic agents and cytotoxic antisera upon these tumor cells will then be studied.

Patient Material:

Patients who have large tumors removed at surgery will be used for these studies.

Major Findings:

Preliminary studies have achieved the successful growth of one out of three human tumors transplanted into rats using the phenomena of acquired tolerance.

Studies with Millipore chambers have confirmed the earlier findings of Algire and others who found that it is possible to grow certain human tumor cells in Millipore chambers placed in laboratory animals.

Significance to Cancer Research:

1. This study, if successful, will demonstrate that the principle of acquired immunological tolerance can also be applied to heterologous transplantation of tumor tissue.

2. New lines of human tumors will be established which can be used to study the effectiveness of various local and systemic chemotherapeutic agents.

3. These human tumor lines can also be utilized for a variety of biochemical, metabolic and immunological studies.

Proposed Course of Project:

This project is just now in the preliminary stages and much more work must be done before the effectiveness of this technique can be evaluated and new tumor lines be established. If this project is successful then the new human tumor lines established can be used for various local and systemic chemotherapeutic studies.

This method, if perfected, would make possible the development of individual tumor sensitivity tests to various chemotherapeutic agents similar to antibiotic sensitivity tests now in use for pathogenic bacteria.

This method could also be used in investigating the cytotoxicity of various antisera to human tumor cells.

NCI-10-773
Serial No.

Part 3.

Project Title: The Effect of Experimental Environmental Factors, Especially Hepatic Trauma, on the Development of Tumor Metastases.

Principal Investigator: Dr. Alan Retik

Project Description:

Objectives:

To determine the effect of stress and liver manipulation upon the metastases of S-91 melanoma and other mouse tumors.

Methods Employed:

Suspensions of S-91 melanoma were injected into the portal vein of mice. After varying periods of time, repeat exploratory laparotomies and liver manipulations were performed as well as other methods of stress, to determine the effect on the incidence and number of liver metastases. The study is in progress now. In addition, groups of mice will be subjected to the various procedures involved in a laparotomy to determine whether these factors influence the development of metastases.

Major Findings:

In a small group of mice, preliminary studies indicate that liver trauma increases the incidence of hepatic metastasis. In a group of 30 mice which were injected intraportally with S-91 melanoma and sacrificed at 3 weeks, no liver metastases were observed. Similarly, in another group of 30 mice which were injected intraportally with S-91 melanoma and sacrificed at 4 weeks, no metastases were observed.

In a group of 40 mice which were injected intraportally with S-91 melanoma, no metastases were noted at laparotomy at 3 weeks. During the laparotomies, the livers were massaged for one minute. At laparotomy, 4 weeks after initial injection, 5 mice were found to have liver metastases.

Significance to Cancer Research:

The above studies, reintroduce the concept of the "dormant cancer cell," which was recently advocated by Dr.'s Edward and Bernard Fisher. One of the questions we are trying to answer is: Can liver trauma with its attendant metabolic alterations cause the growth of tumor cells which have apparently resided dormant in the liver? This is another one of the studies instituted in an attempt to delineate

environmental factors influencing metastic growth of tumor cells.

Proposed Couse of Project:

The course of this study is dependent upon findings of pilot experiments still in progress.

Part B included Yes /XX/ No /___/

NCI-10-773
Serial No.

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Kinsey, David L., Price, Edward B. and Smith, Robert R.: Effectiveness of Chemotherapy in Relation to Extravascular Invasion by Embolic Tumor Cells. Cancer. 13:4, 733-738, 1960.

Potter, John F. and Haverback, Chester Z.: Homotransplantation of Endocrine Tissues in a Diffusion Chamber. Ann. Sug. 151:4, 460-464, 1960.

Arons, Marvin S., Ketcham, Alfred S., and Mantel, Nathan: The Effect of Growth Hormone and ACTH on a Transplanted Tumor. (Accepted for publication in Cancer.)

Honors and awards relating to this project: none

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